Neuroprotective Effect of Vesatolimod in an Experimental Autoimmune Encephalomyelitis Mice Model

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Research Article

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Abstract

Multiple sclerosis (MS) is a chronic, demyelinating autoimmune disease accompanied by inflammation and loss of axons and neurons. Vesatolimod (VES, GS-9620) is a safe and well-tolerated agonist of toll-like receptor 7 with antiviral properties. To further develop possible therapeutic uses of VES, we assessed the effect of VES on MS using an Experimental autoimmune encephalomyelitis (EAE) mouse model which was induced in mice by MOG35–55 injection. Mice were monitored for clinical symptoms daily, and the treatment group was given VES at the onset of illness. The therapeutic effect of VES on EAE inflammation, demyelination, macrophage and T cells infiltration, and microglia activation was evaluated. Autophagy within the spinal cords of EAE mice was also preliminarily assessed. Treatment with VES significantly alleviated clinical symptoms of EAE from day 18 post-immunization and decreased the expression levels of inflammatory cytokines, particularly IL-12 (P40) and Eotaxin, in peripheral blood. It also inhibited demyelination in spinal cords, observed by immunofluorescent staining. Moreover, VES treatment reduced infiltration of CD3 + T cells and CD107b + macrophages, activation of microglia, as well as inhibited the expression of autophagy-related proteins (ATG5, ATG7 and ATG12) in the spinal cords of EAE mice. Our results suggest that VES exhibits protective effects on EAE mice and has the potential to be a novel drug for the treatment of MS.

Introduction

Multiple sclerosis (MS) is a progressive autoimmune and neurodegenerative disease characterized by chronic inflammation, demyelination, and loss of axons and neurons in the central nervous system (CNS) (Baecher-Allan et al. 2018). Pathological processes of MS include breakdown of the blood-brain barrier, multifocal inflammation, oligodendrocyte loss, reactive gliosis, and axonal degeneration (Trapp and Nave 2008; Frohman et al. 2006). Inflammatory CNS lesions associated with MS consist of perivascular and parenchymal infiltrates of lymphocytes and macrophages (Lassmann et al. 2012). In early lesion development, macrophages dominate the infiltrate, followed by T cells and B cells. As the disease advances, diffuse inflammatory T and B cells deeply infiltrate within the CNS, and activated microglia and astrocytes are also present (Dendrou et al. 2015). Although the exact pathological mechanism underlying MS remains unclear, it is generally believed that lymphocyte-mediated immune responses directed against CNS myelin play an important role in disease development. This is evidenced by the extensive lymphocytic infiltration in MS lesions and the effectiveness of immunotherapy treatments. Currently, nearly twenty immunomodulatory drugs have been approved by the FDA to treat MS, however they only partially alleviate disease symptoms and reduce relapse frequency. Therefore, there is an urgent need to develop novel and effective therapeutic MS drugs.

Experimental autoimmune encephalomyelitis (EAE) is an optimal MS animal model because it mimics key characteristics of MS such as chronic inflammation, demyelination, and loss of axons (Correale et al. 2017). Thus, EAE has been frequently used to explore the mechanisms of MS in order to develop new drugs (Duarte-Silva et al. 2019). MS and EAE are both triggered by immune cells, primarily T helper (Th) 1 and Th17 (Jager et al. 2009; Dendrou et al. 2015). Macrophages and microglia also play a role in the
pathogenesis of MS (Alvarez-Sanchez et al. 2020). Throughout the course of MS, autoreactive T cells produce pro-inflammatory cytokines, including IFNγ, IL-17A, and GM-CSF, which promote CNS inflammation (Jager et al. 2009; Yasuda et al. 2019; Baecher-Allan et al. 2018). In recent years, researchers have begun to investigate the relationship between MS and autophagy. Studies have indicated that autophagy plays a pivotal role in inflammation, neurodegeneration, and autoimmune diseases (Li et al. 2020; Deretic and Levine 2018; Frake et al. 2015; Qian et al. 2017; Shibutani et al. 2015).

Vesatolimod (VES, GS-9620) is a small molecule toll like receptor 7 (TLR7) agonist that has been explored as a treatment for hepatitis B virus and HIV-1 due to its antiviral effects. VES has been shown to be safe and well tolerated in participants with hepatitis B virus, hepatitis C virus, and HIV-1 (Janssen et al. 2018; Riddler et al. 2021; Lawitz et al. 2015; Gane et al. 2015). VES directly modulates immune cells leading to direct and indirect production of cytokines (Ram et al. 2020; Lanford et al. 2013; Saitoh et al. 2017). A previous study found that treatment with VES resulted in a sharp reduction in levels of proinflammatory cytokines/chemokines – including IFN-α, IFN-γ, and MCP-1 in mice infected with enterovirus 71 (Zhang et al. 2018). The effect of VES on autoimmune disease pathophysiology is unknown, however toll-like receptors have been implicated in pathogenesis of CNS inflammatory diseases such as MS/EAE through modulation of the production of immunoregulatory mediators (Dieu et al. 2021). For example, the Type I IFN immunoregulatory mediator IFN-β plays a protective role in MS/EAE and has been used as a treatment for MS. Additionally, a previous study reported that imiquimod, a TLR7 agonist, increased IFN-β production and reduced the severity of EAE (O’Brien et al. 2010). However, there have been few relevant studies that have further explored the therapeutic potential of TLRs for EAE.

In this study, we explored the effect of the TLR7 agonist VES on EAE in order to establish evidence to support a new therapeutic direction for MS. Thus, our present study characterized the therapeutic effects of VES using an EAE mice model. We assessed the severity of clinical symptoms, expression of inflammatory cytokines in peripheral blood, demyelination and immunoreaction in the spinal cord, and the expression of autophagy-related proteins in the spinal cord after treatment with VES.

**Methods**

**Animals**

Male C57BL/6J mice were obtained from the Guangdong Medical Laboratory Animal Center. All animals were bred and housed in facilities with controlled temperature and humidity, a 12-h light-dark alternate cycle, and free access to food and water. The use of animals in this study was approved by the Animal Ethics Committee of Shen Zhen university and experiments were performed in accordance with their guidelines.

**EAE Induction**

To induce EAE, male C57BL/6J mice (9 weeks old, 22 ± 2 g) were immunized with 250 µg MOG35-55 peptide (MCE, USA) emulsified in complete Freund’s adjuvant (Chondrex, USA) that contained 5 mg/mL
of heat-killed *Mycobacterium tuberculosis* (H37RA). 400 ng Pertussis toxin (List Biological Laboratories Inc, USA) was administered intraperitoneally at 0 h and after 48 h. Clinical symptoms were scored from the day of immunization as follows (Lu et al. 2020): 0, no symptoms; 0.5, partial limp tail; 1, complete limp tail; 1.5, hind limb ataxia; 2, hind limb paresis; 2.5, partial hind limb paralysis; 3, complete hind limb paralysis; 3.5, hind limb paralysis and fore limb paresis; 4, hind and fore limb paralysis; 5, moribund.

**Experimental design and VES treatment**

VES was prepared for intraperitoneal injection by dissolution in solvent (3% dimethyl sulfoxide + 40% PEG300 + 5% Tween100 + 52% saline). C57BL/6J mice were randomly divided into three groups: control (normal animals, n = 9); EAE (animals immunized with MOG, n = 9); EAE + VES (immunized with MOG and supplemented with vesatolimod, n = 9). At 14 days post-immunization (the onset of EAE symptoms), mice in the EAE + VES group were intraperitoneally injected with vesatolimod (3 mg/kg/day) for 7 days, while mice in the control and EAE groups were intraperitoneally injected with equivalent solvent (3% dimethyl sulfoxide + 40% PEG300 + 5% Tween100 + 52% saline) for 7 days.

**Sample collection**

The weight and symptom scores of the mice in the three groups were recorded for 21 days following immunization. On day 21 post-immunization, mice were sacrificed and peripheral blood, spleen, and lumbar spinal cord were collected. For lumbar spinal cord collection, mice were anesthetized, perfused with 4% paraformaldehyde, and the spinal cord was dissected, fixed with ice-cold 4% paraformaldehyde overnight at 4°C, and dehydrated with 20% and 30% sucrose solution. The lumbar spinal cords were then embedded in O.C.T compound and coronal sections (20 µm) were cut with a freezing microtome. These sections were used for subsequent experiments (histopathology, immunohistochemistry and immunofluorescence).

**Histopathology**

Lumbar spinal cord sections were stained with hematoxylin-eosin (HE) to assess inflammation. First, the sections were degreased with 95% ethanol for 10 min and transferred into hematoxylin solution to stain nuclei for 2 min. The sections were then rinsed in running tap water, immersed in 0.25% ammonia, then redyed with eosin solution for 1.5 min. Finally, the sections were washed in deionized water, dehydrated in alcohol (95%, 100%(I), 100%(II)) for 3 min each, treated with xylene, and sealed with resinene. Images were photographed and analyzed by Olympus Corporation fluorescence microscopy.

**Immunohistochemistry**

For immunohistochemistry experiments, spinal cord sections were incubated with primary antibody dissolved in blocking solution (3% BSA and 0.3% Triton X-100 in PBS) overnight at 4°C. Microglia were labelled with IBA-1 Rabbit mAb (1:100, CST) and macrophages with Purified Rat CD107b (1:30, BD Pharmingen, USA). Next, sections were washed with PBS and incubated with HRP-linked secondary antibody (1:200) at room temperature for 1 h. After an additional PBS wash, a DAB-peroxidase reaction was carried out for 2–8 min. The sections stained with Purified Rat CD107b (1:30, BD Pharmingen, USA)
were counterstained with haematoxylin. All the sections were then dehydrated in alcohol (85%, 95%, 100%
(I), 100%(II)) each for 10 min, treated with xylene, and sealed with resinene. Images were photographed
and analyzed by Olympus Corporation fluorescence microscopy.

**Immunofluorescence**

All antibodies used for immunofluorescence experiments were dissolved in blocking solution (3% BSA
and 0.3% Triton X-100 in PBS). Spinal cord sections were incubated with primary antibody including anti-
Myelin Basic Protein (MBP, 1:300, CST), anti-CD3 (1:150, Abcam), anti-ATG5 (1:200, proteintech), anti-
ATG7 (1:200, proteintech), or anti-ATG12 (1:100, proteintech) for 4°C overnight. The sections were then
washed with PBS and incubated in Alexa Fluro-conjugated secondary antibodies (1:400, Invitrogen) for 1
h at room temperature. After a PBS wash, the sections were transferred onto slides which were subjected
to the DAPI nuclear marker. T cells were labelled with anti-CD3 and demyelination with anti-MBP. Images
were photographed and analyzed by Carl Zeiss Suzhou fluorescence microscopy.

**Western blot**

Isolated lumbar spinal cords were homogenized in cold RIPA buffer (beyotime, Shanghai, China)
supplemented with 1mM PMSF inhibitor (beyotime, Shanghai, China). Protein concentration was
measured by using the Omni-Easy™Instant BCA Protein Assay Kit (Epizyme, Shanghai, China). Proteins
(20–30 µg) were separated on 12.5% SDS-PAGE gels by electrophoresis then transferred to PVDF
membranes and blocked at room temperature with Protein Free Rapid Blocking Buffer (Epizyme,
Shanghai, China). The membranes were incubated at 4°C overnight with primary antibodies for anti-Iba1
(1:500, CST), anti-MBP (1:1500, CST), anti-ATG5 (1:1000, proteintech), anti-ATG7 (1:600, proteintech), and
anti-GAPDH (1:1500, CST). Secondary antibodies, either HRP-linked anti-rabbit IgG (1:2500, CST) or anti-
mouse IgG (1:2500, CST), were then added to the membrane for 1 h at room temperature. Protein bands
were visualized by SuperSignal™ West Pico plus Stable Peroxide (thermos scientific, USA). Protein
signal was quantified using ImageJ analysis software.

**LiquiChip Luminex assay**

Peripheral blood was collected and placed at room temperature for 20 minutes to clot naturally. The
blood samples were then centrifuged at 1000 g for 10 min at 4°C and the upper layer of serum was
transferred into 1.5 mL frozen storage tubes and stored at −80°C. Liquid bead suspension chips (Bio-Plex
Pro Mouse Cytokine Grp I Panel 23-plex) were customized by Shanghai Wayen Biotechnologies (China) to
detect the expression of related cytokines.

**Statistical analysis**

GraphPad Prism 9 was used for statistical analyses. Results are expressed as mean ± standard error of
the mean (SEM). One-way ANOVA followed by Dunnett’s posttest was used to analyze weight, symptoms
scores, and the expression of cytokines and proteins. P < 0.05 was considered significant.

**Results**
VES ameliorates the clinical symptoms of EAE

To explore the therapeutic potential of VES on EAE, we recorded clinical scores and body weight of mice in three groups – a control group, EAE group, and an EAE group treated with VES – daily for 21 days post-immunization. Symptoms of EAE started on day 15 post-immunization (Fig. 1A, 1C), after which there was a rapid increase in the clinical scores among mice in the EAE group. However, the mean clinical score of VES-treated mice was significantly lower on day 18 post-immunization, and on all subsequent days, relative to the untreated EAE group (Fig. 1C). To evaluate the severity of EAE symptoms, we compared both the cumulative and maximum scores of the three groups. As shown in Fig. 1D and 1E, the cumulative and maximum scores of VES-treated mice were significantly lower than those of EAE mice. Additionally, EAE mice weight decreased from day 15 post-immunization relative to the control mice. After VES treatment (day 14), weight began to recover by day 16, and by day 20 the VES-treated mice had a significantly higher average weight compared to the EAE group (Fig. 1B).

VES inhibits inflammation in the peripheral blood and spinal cords of EAE mice

EAE is an autoimmune disease associated with severe neuroinflammation. Therefore, we measured whether VES treatment decreases inflammatory cell infiltration or inflammatory cytokine levels in peripheral blood. HE staining revealed that EAE mice exhibited extensive inflammatory cell infiltration in the white matter of the spinal cord, whereas VES + EAE mice had only mild infiltration (Fig. 2A). In addition, expression levels of proinflammatory cytokines (such as IL-17A, IL-6, G-CSF, IL-12 (p40), and Eotaxin) were dramatically higher in the serum of EAE mice relative to control mice (Fig. 2B, C, D, E, F). After VES treatment, expression levels of both IL-12(P40) and Eotaxin decreased significantly (Fig. 2E, F).

VES reduces demyelination in the spinal cord of EAE mice

To further confirm the therapeutic effect of VES in EAE, we measured demyelination in mouse spinal cords via immunofluorescent staining of myelin basic protein (MBP), an important component of myelin. We observed diffuse demyelination in the white matter of spinal cords in EAE mice (Fig. 3A). Following VES-treatment, the area of demyelination was clearly reduced compared to the EAE group. Additionally, protein quantification revealed significantly higher levels of MBP in the VES-treated group compared to the EAE group (Fig. 3B, C).

VES represses immunoreaction in the spinal cords of EAE mice

T cell-mediated neuroinflammation is observed during the onset of EAE, while macrophages play an important role in EAE progression, thus we measured infiltration of T cells and macrophages in spinal cords using immunofluorescent staining of CD3 and CD107b, respectively. As shown in Fig. 4A and B, a large number of T cells and macrophages were observed in the spinal cord white matter in EAE mice, while only mild infiltration was observed in VES + EAE mice.
Microglia are the resident macrophages of the CNS, therefore to compare microglia levels across treatment groups, we measured the microglia-specific protein Iba-1 using immunofluorescent staining and protein quantification (Fig. 4C, F, G). Immunofluorescent staining of Iba-1 showed dramatic microglia activation and proliferation in the whole lumbar spinal cords of EAE mice compared to control mice. Moreover, the amount of activated microglia was markedly lower in VES + EAE mice relative to the untreated EAE group (Fig. 4C). There were also significantly different protein levels of Iba-1 across the three groups, with the highest levels observed in the untreated EAE mice (Fig. 4F, G).

Comparison of spleen weights across the three groups indicated that VES treatment may promote splenic hyperplasia in mice (Fig. 4D). Statistical analysis confirmed that there were significant differences in spleen weight among three groups (Fig. 4E).

**VES inhibits autophagy in the spinal cord of mice with EAE**

Autophagy regulates inflammation in various diseases and is associated with autoimmune diseases. To examine the effect of VES on autophagy in EAE, we determined the location of autophagy-related proteins in mouse spinal cords using immunofluorescence and quantified their expression in the three experimental groups. In comparison with control mice, EAE mice had greater immunofluorescent signals of proteins ATG5, ATG7 and ATG12 in the white matter of the spinal cord. After VES treatment, the fluorescent signal of these proteins significantly decreased (Fig. 5A). Quantification of ATG5 and ATG7 in spinal cords revealed significant differences among the three groups (Fig. 5B, C, D, E).

**Discussion**

MS is an autoimmune and neurodegenerative disease that mainly affects young adults, often causing disability, that is increasing in prevalence across the globe (Hauser and Cree 2020). Early symptoms include difficulty walking, fatigue, blurred vision, and numbness, tingling, and/or weakness in the limbs or other parts of the body. Progressive symptoms include muscle stiffness, cognitive deficits, and urinary problems (Gu 2016). The current primary therapeutic approaches for MS mainly target T lymphocytes, B lymphocytes, microglia, and macrophages in order to alleviate inflammation and promote the regeneration of the myelin sheath (Faissner et al. 2019). Moreover, disease-modifying therapies (DMTs) can reduce relapse rates by exerting immunosuppressive or immunomodulatory effects. Despite many effective drugs available, no current treatment completely cures MS.

VES is a safe and well-tolerated toll-like receptor 7 agonist that can be administered orally. Previously, Eric Lawitz et al. reported that VES exhibits dose-linear pharmacokinetics with an 18 h median half-life in the plasma of patients with chronic hepatitis C (Lawitz et al. 2015). In this study, we investigated the therapeutic effect of VES on EAE, a model that closely resembles MS. Based on previous studies, including Kyle E. Korolowizc et al. (Korolowizc et al. 2019), we chose to administer 3mg/kg of VES to EAE mice intraperitoneally each day. It has been previously reported that C57BL/6 EAE mice have more severe tissue damage in the spinal cord than in the brain (Gogoleva et al. 2018). We further determined by HE pre-dyeing that the most severe tissue damage is present in the lumbar segment of spinal cord in these
mice. Therefore, the lumbar spinal cord was isolated and used for experimental analysis in this study. Overall, our results suggest that VES alleviates clinical symptoms and inflammation, suppresses infiltration of macrophages and T cells, reduces demyelination, and inhibits the activation of microglia.

Macrophages and microglia are considered to play an important role in the pathogenesis of MS (Weng et al. 2018; Franco and Fernandez-Suarez 2015). These cells are divided into M1 and M2 subtypes according to their role in inflammation. Activated M1 cells exhibit pro-inflammatory properties in EAE, whereas M2 cells play an anti-inflammatory role and thus alleviate symptoms of EAE (Chu et al. 2018). Excessive activation of microglia can induce a severe inflammatory response in the CNS through production of a large amount of pro-inflammatory cytokines and chemokines. This promotes recruitment of peripheral immune cells into the CNS, resulting in oligodendrocyte death and demyelination neuroaxonal injury in EAE/MS (Mishra et al. 2014; Zhou et al. 2015). Therefore, preventing microglia overactivation and macrophage infiltration, or promoting M2 subtype polarization, are hypothesized to be therapeutic strategies for MS (Schilling et al. 2006; Cai et al. 2021; Chen et al. 2019; Weng et al. 2018). Numerous studies have shown that suppressing macrophage infiltration and blocking microglia activation alleviate the severity of EAE (Schilling et al. 2006; An et al. 2020; Weng et al. 2021; Zha et al. 2021). In this study, we demonstrated that VES significantly attenuates macrophage infiltration and microglia activation. As shown in Fig. 4, treatment with VES reduced the expression level of Iba-1 and MBP, attenuated microglia activation, and suppressed macrophage infiltration in the spinal cords of VES-treated EAE mice relative to EAE mice. Together our results suggest that the therapeutic potential of VES in EAE may be partially due its ability to inhibit microglia activation and macrophage infiltration.

MS pathogenesis is mediated primarily by T cells, particularly CD4+ T and CD8+ T cell subtypes. Th1 CD4+ T cells secrete large amounts of IFN-γ which activates macrophages (Jager et al. 2009), and Th17 CD4+ T cells produce IL-17A, a major mediator of tissue inflammation in MS/EAE. Previous studies have reported that reducing infiltration of all T cell types alleviates the severity of EAE (Zhang et al. 2016; Li et al. 2018). Our results show that treatment with VES blocks T cells infiltration in the spinal cord of mice with EAE, and this suppression of infiltration likely contributes to the therapeutic properties of VES.

Demyelination is one of the most common characteristics of EAE. In this study, we evaluated spinal cord demyelination by staining for and measuring protein expression levels of MBP. Figure 2 illustrates that demyelination widely occurs in the white matter of EAE mice spinal cords, and that T cells and microglia contributed to this process. Moreover, we found that VES suppressed T cell infiltration and microglia activation which reduced further damage to the spinal cord. Collectively, VES effectively alleviates demyelination of the damaged spinal cord in EAE mice. IL-1β, IL-6, and IL-23 induce Th17 cell pathogenicity and elicit EAE (Ghoreschi et al. 2010; Yasuda et al. 2019), however blocking the IL-6 signal can reduce T cell infiltration and ameliorate EAE (Ji et al. 2019). IL-17A, which is secreted by Th17 cells, is considered a central mediator of autoimmunity in EAE. A major function of Th17 cells is the induction of granulocyte-colony stimulating factor (G-CSF) expression to activate and mobilize neutrophils. Prior research has reported that G-CSF receptor (G-CSFR) deficiency suppressed the accumulation of circulating myeloid cells and improved the clinical course of EAE (Rumble et al. 2015). In our study, we
found that levels of IL-17A, IL-6, and G-CSF were significantly higher in the serum of EAE mice than in control mice, and treatment with VES reduced their expression (Fig. 2B, C, D). IL-12 is a heterodimeric molecule that stimulates natural killer (NK) cells and T cells, including Th1 cells, to produce IFN-γ(Segal 2010). Th1 cells are suggested to be critical mediators of EAE, as a previous study found that EAE did not occur in the absence of the IL-12(p40) subunit(Baecher-Allan et al. 2018). Eotaxin (CCL11) is a small cytokine belonging to the CC chemokine family that selectively recruits eosinophils. It has been reported that Eotaxin exacerbates inflammation in neuromyelitis optica spectrum disorder(Tong et al. 2018). Similarly, we found that the levels of IL-12(p40) and Eotaxin in the serum of EAE mice were significantly higher than in control mice, and that administration of VES significantly reduced these levels (Fig. 2E and F). Collectively, our study shows that the therapeutic effects of VES may be partially due to lower expression levels of proinflammatory cytokines, such as IL-17A, IL-6, G-CSF, IL-12(p40) and Eotaxin, in serum.

Autophagy is a cellular process that deals with damaged organelles, denatured proteins, and invading pathogens through the lysosomal degradation pathway(Qian et al. 2017; Galluzzi and Green 2019). It is established that autophagy is linked to the progress of MS/EAE(Liang and Le 2015). Several studies have shown that regulation of the autophagy signaling pathway can alleviate symptoms of EAE(Yuan et al. 2019; He et al. 2021; Li et al. 2019; Duarte-Silva et al. 2021; Dang et al. 2014; Yang et al. 2021). However, little is known about the exact role of autophagy in MS. In fact, whether autophagy leads to cell death or provides a neuroprotective effect in MS remains controversial. Our study initially explored the expression of autophagy-related proteins, including ATG5, ATG7 and ATG12, in the spinal cord. ATG5, ATG7 and ATG12 are essential for the induction of autophagy. Atg7 catalyzes Atg5–Atg12 conjugation, which then forms a multimeric complex with ATG16L and interacts with the outer membrane of the extended phagocytic vesicle(Kaur and Debnath 2015). We found extensive immunofluorescent signals of ATG5, ATG7 and ATG12 in white matter lesions within spinal cords of EAE mice (Fig. 5A); in fact, EAE mice had significantly higher ATG5 and ATG7 expression compared to control and VES-treated mice. Consequently, we speculate that extensive autophagy occurred at these EAE-induced spinal cord lesions, and that the therapeutic effects of VES might partially be due to inhibition of the Atg5–Atg12–Atg16L signaling pathway.

Conclusion

Our study demonstrates for the first time that administration of VES reduces the clinical symptoms caused by EAE, decreases the level of proinflammatory cytokines in serum of EAE mice, and inhibits the demyelination, immunoreaction, and autophagy in spinal cord of EAE mice. These effects of VES likely occur through inhibition of both microglia activation and macrophage and T cell infiltration in the spinal cord. Further studies are required to determine the mechanisms involved in the observed clinical effect of VES in the EAE model. Ultimately, VES is a promising candidate for future trials in MS cohorts.

Declarations
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Author Contributions: All authors contributed to the study. The study conception and design were performed by Jian Zhang, Xian Jiang and Shuhua Mu. Material preparation, data collection and analysis were performed by Xian Jiang, Yifan Song, Xiao and Jie Fang. The first draft of the manuscript was written by Xian Jiang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

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References


viral recognition results in focal type I interferon secretion by dendritic cells. Nature Communications 8. doi:10.1038/s41467-017-01687-x


Figures

Figure 1

VES improves the body weight and ameliorates clinical symptoms of EAE mice. **A** Clinical symptom of EAE mice. The black arrow points to the paralyzed area of the mouse. **a** complete limp tail; **b** partial hind limb paralysis; **c** complete hind limb paralysis. **B** body weight of mice. **C** clinical score of mice. **D** the statistical graph of the maximum score (n=9). **E** the statistical graph of the cumulative score. n = 9 mice / group. For **B** and **C**, *p<0.05 and **p<0.01, compared between the control and EAE groups; #p < 0.05 and ##p < 0.01, compared between the EAE and EAE+VES groups. For **D** and **E**, *p < 0.05, and **p < 0.01, compared to each other.
Figure 2

VES inhibits inflammation of EAE mice. A representative image of HE-stained sections of lumbar spinal cord. Scale bar, 200 μm, 100 μm. Expression level of cytokines in peripheral blood including B IL-17A; C IL-6; D G-CSF; E IL-12 (p40); F Eotaxin (n=3). Data are presented as mean ± SEM; compared with the EAE group, *P<0.5.
Figure 3

VES alleviates demyelination in the spinal cord of EAE mice. **A** Representative immunofluorescent staining sections of MBP in lumbar spinal cord. Scale bar, 100 μm. **B** Representative western blot images of MBP in lumbar spinal cord tissues. **C** Columns represent normalized densitometric analyses (n=3). Scale bar, 200 μm, 100 μm. Data are presented as mean ± SEM; compared with the EAE group, *P < 0.05.
VES represses immunoreaction in spinal cord of EAE mice. A Representative immunofluorescent staining sections of CD3. B Representative immunohistochemical staining sections of CD107b, nuclei were counterstained with hematoxylin. C Representative immunohistochemical staining sections of Iba-1 in lumbar spinal cord. Scale bar, 200 μm, 100 μm, and 50 μm. D The image of spleen of the mice in three groups. E The bar graph of spleen weight. F Representative western blot images of Iba1 and corresponding GAPDH in lumbar spinal cord tissues. G Columns represent normalized densitometric analyses (n=3). Data are presented as mean ± SEM; compared with each group, **P < 0.01.
Figure 5

VES inhibits autophagy in spinal cords of EAE mice: A Representative image of immunofluorescence for ATG5, ATG7 and ATG12. Scale bar, 100 μm. B and D the protein level of ATG5 and ATG7 in lumbar spinal cord was analyzed by western blot. C and E Columns represent normalized densitometric analyses for the western blot band of B and D (n=3). Data are presented as mean ± SEM; compared with each group, *P<0.05, **P < 0.01.

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