Mediation of the SPHK1/S1P Pathway Following Vitamin D Treatment of MS/EAE

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Research Article

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Abstract

Sphingosine-1-phosphate (S1P) is a bioactive lipid that distributed in a wide variety of body tissues, especially inflammatory tissues. We observed that S1P plays an important role in the pathogenesis of Experimental allergic encephalomyelitis (EAE), the animal model of Multiple sclerosis (MS). Additionally, Vitamin D is observed to be a key determinant in the regulation of S1P. We monitored the effects of Vitamin D on Sphingosine kinase type 1 (SPHK1), an important synthetase associated with S1P. The associated results suggest a reduction in SPHK1 in the spinal cords of EAE rats whose diets supplement with Vitamin D. Many of the biological functions that are traditionally associated with Vitamin D is mediated through the Vitamin D receptor (VDR). Indeed, these interactions play a major role in the regulation of transcription of many target genes. As part of this analysis, we revealed the relationship between VDR and SPHK1. This is performed using over-expression experiments and observations relating to the interference effects of VDR on SPHK1. Our results verify that expression of the factor S1P, which is associated with EAE, is caused by alterations in SPHK1 levels. This relationship is regulated by VDR. Consequently, immune regulatory mechanisms associate with Vitamin D adjuvant therapy, used for the treatment of MS, may be involved in the regulation of SPHK1. This regulatory role may ultimately modulate the levels of S1P in cells. This report focuses on the relationship between SPHK-S1P and Vitamin D and provides a potentially novel approach for the treatment of MS.

Introduction

MS is an autoimmune disease associated with inflammation of the central nervous system [1]. Concomitant with inflammatory reactions that occur as a consequence of neural degenerative diseases [2], patients typically display symptoms such as optic neuritis, limb paralysis, sensory disorders, balance and coordination difficulties, and cognitive and sleep disorders [1, 3–5]. The etiology associated with MS is still unclear [1]. Up until now, a curative agent has not been reported for MS. Commonly used treatment regimens have predominantly focused on immune system regulation (e.g. interferon-β) and related therapies (FTY-720, etc) [6–8]. Although these drugs can facilitate disease symptom alleviation and a reduction in disease recurrence rates [6–8], the overall performance of these treatments is questionable. The vast majority of these treatments have serious side effects [9–13]. Therefore, there is an urgent requirement to source new treatments in relation to the safe and effective treatment of MS and related diseases [1].

Vitamin D is best known as a calcium homeostasis modulator. However, emerging research has shown that low levels of Vitamin D increase the risk of developing MS. 1,25-dihydroxy Vitamin D3 is the biologically active form of Vitamin D in the body. In rats, it has been shown that Vitamin D can delay progression in the animal model of MS. However, Vitamin D cannot cure the disease or terminate disease progression. Therefore, the application of Vitamin D in MS and EAE treatment is limited[14, 15].

S1P is a bioactive lipid and is closely involved in tumor signaling pathways, anti-apoptosis effects, hypoxia tolerance, abnormal transcriptional regulation, angiogenesis and tumor metastasis and invasion.

In addition, a recent study has shown that the regulation of inflammation plays a considerable role in all stages of tumor occurrence and development [16–18]. SPHK1 is capable of facilitating catalytic conversion of SPH into S1P. SPHK1, is predominantly distributed in the cytoplasm[19]. SIP can also be re-located to the outside of the cell, following activation of the S1P receptor(S1PR). Indeed, this receptor plays an important role in the activation of a series of downstream signaling pathways [20]. It is also used as a secondary messenger and directs its effects on a number of differential targets in the cell [21].

In recent years, a large number of studies have shown that S1P in combination with S1PR can result in the generation of reactive astrocytes [22–27], as well as triggering neuronal apoptosis. Interestingly, experiments have shown that large numbers of reactive astrocytes are common in the vicinity of demyelinating lesions associated with MS patients and EAE animals [26]. These cells release inflammatory cell factors that result in the exacerbation of MS and EAE inflammation [27]. Additionally, neuronal apoptosis is closely related to the activation of astrocytes [28, 29]. This suggests that the interaction of S1P and S1PR can result in the generation of reactive astrocytes, thereby promoting MS/EAE. Ultimately, S1P is likely to be associated with MS in vivo.

As part of this analysis, we observed for the first time that Vitamin D results in a reduction in EAE mouse S1P levels, suggesting that Vitamin D may inhibit S1P/S1PR interaction, thereby relieving the symptoms associated with EAE. In this study, we also observed that EAE rats who were administered Vitamin D contained a significant reduction in S1P levels in the spinal cord when compared with rats whose diets were not supplemented with Vitamin D. This analysis was facilitated using high performance liquid chromatography (HPLC) [30].

In view of this result, we hypothesize that Vitamin D reduces immune reactions caused by S1P. This has the effect of delaying EAE and reducing the symptoms associated with the EAE disease condition by lowering EAE mouse S1P levels. In the body, Vitamin D regulates the transcription of target genes following interaction with the VDR [31]. In this report, we observed the relationship between VDR and SPHK1 following over-expression of and interference with VDR. Our results verified that expression of the inflammatory factor S1P in MS and EAE is caused by a change in SPHK1 levels. This reaction is regulated by VDR. Therefore, the immune regulatory mechanism associated with MS-related Vitamin D adjuvant therapy is likely to regulate SPHK1 activity resulting in a reduction in S1P levels. It is likely that if Vitamin D is administered in conjunction with other anti-S1P drugs, there is an increased chance of MS/EAE prevention and treatment.

Materials And Methods

EAE in Rats

All experimental protocols involving animals were approved by the Animal Care Committee of Shandong University . Eight-week-old male Lewis rats were injected subcutaneously in two hind foot pads with 100 µl immunization emulsion, and 50 µl per pad. Control animals were injected with parallel emulsion without the MBP component. Details have been described previously by Zhu Y. et al(2014) [30]. The rats

were first separated into two groups based on their EAE status: control (C) and EAE (E). The rats from EAE groups were fed with Vitamin D (V) or without Vitamin D. This resulted in four groups of rats: C, VC, E and VE. The synthetic diet contains all essential nutrients, except Vitamin D. The diet containing Vitamin D supplementation was prepared with 3 μg/ day added for each rat. Fresh food was provided to rats every other day for 2 weeks prior to euthanasia.

Cell Culture

PC12, a common nerve cell line derived from *Rattus norvegicus*, was obtained from ATCC. PC12 cells were cultured in 75 cm^2 cell culture flasks or 6-well cell culture plates (Fisher Scientific) using Dulbecco's Modified Eagle's Medium (DMEM) (Hyclone) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin and streptomycin. The cultures were stimulated with different concentrations of 1,25-$(OH)_2D_3$. Cultures were maintained at 37°C in a humidified incubator with 5% CO_2 (Thermo).

Measurement of S1P

Tissue homogenates were prepared from 30 mg of excised spinal cord using a homogenizer in 100 μl of 25 mM HCl/1 M NaCl, and the S1P concentrations in the spinal cord homogenates were measured using HPLC.

Cells (1×10^6) were diluted with PBS (pH 7.2–7.4). The cells were lysed following repeated freeze/thaw cycles to release intracellular components. The S1P concentrations in the PC12 cells supernatant were measured with enzyme-linked immunosorbent assay (ELISA) kits (ShangHai QiaoDu Biotechnology) according to the manufacturer's instructions.

Quantitative Real-Time PCR

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) synthesis was performed using EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TRANS). These reactions were performed in accordance with the manufacturer's instructions. Quantitative Real-time PCR amplification was performed on the CFX96 Real-Time PCR System (Bio-Rad) using the SYBR Green PCR Master Mix (Roche, Germany). The Real-Time PCR used the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s. GAPDH, a housekeeping gene, was used as an internal control. The relative concentration of SPHK1 was calculated using the formula $x = 2 - \Delta\Delta CT$.

Western Blotting

To assess SPHK1 expression in EAE rats, we collected spinal cord samples from both EAE and normal rats to investigate the difference in protein expression levels between the different groups. PC12 Cells and spinal cords were lysed in RIPA Lysis Buffer (Beyotime, Shenzhen, Guangdong, China) followed by homogenization and centrifugation steps. Protein concentrations were measured using the BCA protein assay (Beyotime). Equal amounts of protein were electrophoresed using 10% SDS-polyacrylamide gel

electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The membrane was subsequently blocked with 5% non-fat dry milk in Tris-buffered saline with 0.1% Tween 20 (TBS-T) at room temperature for 1.5 h. The membranes were incubated overnight with SPHK1 primary antibody (1:1,000) at 4°C. Next, the membranes were washed with TBS-T and incubated with horseradish peroxidase labeled anti-rabbit IgG (1:5,000, ZSGB-BIO, Beijing, China). The membranes were subsequently washed three times with TBS-T buffer, and the immunoreactive complexes were visualized using the Lumina Enhanced Chemiluminescent Kit (Millipore, Billerica, MA, USA). Finally, luminescent products were visualized using photographic film. Actin was used as an internal standard.

RNA expression

A VDR expression plasmid (GV230, Genechem, Shanghai, China) was injected into PC12 cells facilitating stable expression of VDR. Alternatively, a control plasmid (NC) was injected into PC12 cells. PC12 cells were cultured in 6-well plates and transfected when 70–80% confluence was reached. Transfections were performed using the Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. VDR expression, facilitated by the introduction of the plasmid, was verified by Western blotting.

RNA Interference

In order to facilitate VDR expression interference, 19-mer siRNA oligos □GGAAGTACAGGGAGCTATT□were designed and synthesized (Vdr-RNAi, Genechem, Shanghai, China). A Lentiviral expression vector (GV118, Genechem, Shanghai, China) and control Lentiviral expression vector were used to ensure transfection efficiency. The Lentiviral expression vector was injected into PC12 cells, thereby stably interfering with VDR expression. The PC12 cells used in this experiment were grown in 6-well plates and transfected at 30–50% confluence. Transfections were performed according to the manufacturer's instructions. VDR interference, facilitated by introduction of the Lentiviral vector, was verified by Western blotting.

Results

Vitamin D supplementation delays the onset and initial severity of EAE rat model

We established the EAE model in our laboratory, Rats were fed without or with Vitamin D supplementation for control rats (C vs VC) and EAE rats (E vs VE). From the first day after MBP injection, the weight of rats in the control group increased continuously. However, the EAE rats began to lose weight on the day 7 after MBP injection, and gradually recovered weight on the day 16, but the weight change of rats from VE was significantly less after MBP injection(Fig. 1a). We calculated the severity score for 2 weeks following EAE induction. The first sign of EAE appeared at about day 7 in E rats, but was delayed more than a day in VE rats (Fig. 1b), thus verifying a protective effect of vitamin D in the EAE model.

The levels of SPHK1 are increased in spinal cord during EAE, and this increase is reduced upon Vitamin D supplementation

Spinal cord samples were isolated from EAE rats. We performed western blots to determine whether SPHK1 might also be modulated in EAE by Vitamin D, the associated results suggest a reduction in SPHK1 in spinal cords of EAE rats whose diets were supplemented with Vitamin D(Fig. 2a, b). Based on this finding, We used Quantitative Real-Time PCR to test SPHK1 related to S1P in the spinal cords of EAE rats with (VE) or without (E) Vitamin D supplementation(Fig. 2c), we observed significant changes in SPHK1 mRNA levels. These results suggest that the levels of SPHK1 are increased in spinal cord during EAE, and this increase is reduced upon Vitamin D supplementation.

The Levels of S1P are increased in the Spinal Cord During EAE, and this increase is reduced upon Vitamin D supplementation

Spinal cord was obtained from EAE rats (E vs VE) and was analyzed by HPLC (Fig. 3a) to determine S1P concentrations. S1P concentrations in the spinal cords of EAE rats were higher during E group compared with the VE group(Fig. 3c). Furthermore, the increase in S1P during the VE group was not as dramatic for rats fed with vitamin D.

SPHK1 and S1P are decreased in PC12 cells stimulated with different concentration of 1,25-$(OH)_2D_3$

Based on the findings that the levels of SPHK1 are reduced upon Vitamin D supplementation ,we sought to determine whether SPHK1 are changed in the PC12 cells stimulated with different concentration of 1,25-$(OH)_2D_3$ for 24, 48, or 72 h(Fig. 4a), the PC12 cells was obtained from the six different groups stimulated with different concentration of 1,25-$(OH)_2D_3$(0 nmol/L, 6.25 nmol/L, 12.5 nmol/L, 25 nmol/L, 50 nmol/L, 100 nmol/L). As part of this study we observed changes in SPHK1 and found that when high concentration of 1,25-$(OH)_2D_3$ were applied, SPHK1 protein levels and mRNA levels decreased(Fig. 4b, c). We conclude that Vitamin D plays an important role in the regulation of SPHK1 synthetase. Following ELISA-based analysis performed on the associated S1P, we observed that S1P levels are changed in PC12 cells stimulated with different concentration of 1,25-$(OH)_2D_3$ for 24, 48, or 72 h (Fig. 4d), and found that when high concentrations of 1,25-$(OH)_2D_3$ were applied, S1P levels decreased. The results confirmed that Vitamin D is affected following the synthesis S1P.

Vitamin D regulates the expression of SPHK1 following binding of VDR.

Many of the biological functions that are traditionally associated with Vitamin D are mediated through the VDR. As part of this analysis, we revealed the relationship between VDR and SPHK1.This was performed using over-expression experiments and observations relating to the interference effects of SPHK1 on VDR. A VDR expression plasmid(GV230) was injected into PC12 cells facilitating stable expression of VDR, PC12 Cells were stimulated for 24, 48, or 72 h without and with 1,25-$(OH)_2D_3$ (100 nmol/L) following transfection with the VDR plasmid (GV230) or control (NC) plasmid. Following western blots(Fig. 5a), we observed the levels of SPHK1 are reduced in PC12 Cells stimulated with 1,25-$(OH)_2D_3$ (100 nmol/L), the levels of SPHK1 are not change significantly in PC12 Cells stimulated without 1,25-$(OH)_2D_3$ (100 nmol/L). The over-expression experiments gray value for each band was

calculated(Fig. 5b). Following ELISA-based analysis performed on the associated S1P(Fig. 5c), we observed that S1P levels are changed in PC12 cells stimulated with 1,25-$(OH)_2D_3$ for 24, 48, or 72 h, and found that in cells which were following transfection with the VDR plasmid (GV230) S1P levels decreased significantly. VDR interference in PC12 cells was verified by Western Blot analysis following injection of the Lentiviral expression vector and control Lentiviral expression vector(Fig. 5d). We observed the RNA interference effectively reduced VDR protein levels compared to the control (NC) and found that the levels of SPHK1 did not change significantly in PC12 Cells stimulated with 1,25-$(OH)_2D_3$ (100 nmol/L) when reduced VDR protein levels. The interference gray value for each band was calculated(Fig. 5e, f). Following ELISA-based analysis performed on the associated S1P(Fig. 5g), we observed that S1P levels are changed in PC12 cells stimulated with or without 1,25-$(OH)_2D_3$ (100 nmol/L) when reduced VDR protein levels. These results support the regulation of Vitamin D on SPHK1 expression is relied on its combination with VDR.

Discussion

In recent years, important physiological functions associated with S1P have been elucidated. S1P has been found to be an important intracellular secondary messenger. It has also been observed to bind to specific receptors on the surface of cells, thereby playing an important role in a number of critical biological reactions [19]. Recent evidence has suggested that S1P is important in cell proliferation and in the optimal functioning of the cardiovascular system [32–34].

Vitamin D is a key effector in the regulation of bodily calcium homeostasis. However, in recent years, studies have shown that Vitamin D plays an important role in regulating the immune system [31], thereby assisting in protection against immune system diseases [35, 36]. Indeed, recent research has focused on one such disease, MS. MS is an autoimmune disease, and the relationship between Vitamin D and MS occurrence has become a relatively important area of focus from a research perspective [37]. Indeed, the relationship between MS and Vitamin D was recently studied as part of an epidemiological survey. The survey revealed there to be a close correlation between MS and sun exposure [38]. Sun exposure is a key catalyst required for the synthesis of Vitamin D in the body. Interestingly, the former study suggests that sun exposure, while influencing the levels of Vitamin D in the body, also plays a role in the occurrence of MS [39]. Subsequent experiments demonstrated that MS patients have reduced levels of 25-$(OH)_2D_3$ [40, 41]. Other studies have shown that Vitamin D levels in the body are associated with the occurrence of MS [42–44]. The same effect was observed in relation to EAE (animal model of MS), with experiments suggesting that dietary supplementation with Vitamin D can effectively delay and alleviate EAE [37]. Taken together, these results provide strong evidence that there is a significant correlation between Vitamin D and MS disease occurrence and development [30, 45]. We inferred from these results that Vitamin D is involved in S1P regulation, and thereby preventing initiation of the S1P cascade. In effect, this results in preventing MS [46]. SPHK inhibitors are the preferred therapeutic agent for the treatment of MS. This inhibitor results in an alteration in S1P levels, thereby affecting the concentration of S1P-related enzymes following stimulation with Vitamin D in treated cells. The synthesis and decomposition of S1P

is controlled by a variety of enzymes [47]. As part of this analysis we looked at the effects of 1,25-$(OH)_2D_3$ at the cellular level using real-time PCR. Gene expression levels associated with SPHK1 were analyzed and the results illustrated that Vitamin D directly controls expression of SPHK1 following stimulation with 1,25-$(OH)_2D_3$. ELISA was used to analyze cell extracts following incubation with different concentrations of 1,25-$(OH)_2D_3$. As part of this study we observed changes in S1P and found that when high concentrations of 1,25-$(OH)_2D_3$ were applied, S1P levels decreased. The results confirmed that Vitamin D is affected following the synthesis of S1P. Our experiments also verified that SPHK1 levels varied between tissues extracted from EAE rats. The results of the analysis confirmed that SPHK1 expression levels were higher in EAE rats than in normal rats. Consequently, we conclude that Vitamin D plays an important role in the regulation of SPHK1.

In recent years, the role of Vitamin D has become the focus of studies relating to the treatment and prevention of MS [48]. Indeed, the appropriateness of Vitamin D as a MS immunomodulatory drug is now being tested in the second phase of clinical trials [49]. However, the role of Vitamin D in relation to MS is unclear. The pharmacological effects associated with the vitamin are unstable [50] and further studies are required to elucidate the relationship between Vitamin D and MS. As a consequence of this study, we observed that the interaction between Vitamin D and VDR can affect the associated levels of SPHK1. The latter affects the synthesis of S1P, thereby regulating interactions between S1P and its receptor, these interactions influence S1P-S1PR pathways and related biological activities [51–53].

In this experiment, we observed that SPHK1 levels were increased in EAE rats spinal cords. SPHK1 was decreased in the spinal cords of EAE rats following Vitamin D supplementation. Upon complexing of Vitamin D with VDR, SPHK1 synthesis is reduced. This results in a reduction in S1P synthesis, which facilitates the induction of EAE. VDR, as a nucleoprotein, is mediated by the presence of 1,25-$(OH)_2D_3$ (which plays a role in many important biological activities). Following the use of over-expression experiments and observations relating to the interference effects of SPHK1 on VDR, we found that Vitamin D regulates the expression of SPHK1 following binding of VDR. However, the exact mechanism of this activity is still relatively unclear and we hope to further research. This study suggests that the use of Vitamin D supplements in the clinical treatment of MS patients will facilitate adequate disease recovery.

Abbreviations

S1P Sphingosine 1-phosphate

SPH Sphingosine

SPHK Sphingosine kinase

S1PR Sphingosine 1-phosphate receptor

MS Multiple Sclerosis

EAE Experimental allergic encephalomyelitis

VDR Vitamin D receptor

Declarations

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Authors' contributions

D. Y. initiated the research. D. Y. and Z. W. designed all of the experiments. D. Y. supervised the overall project design and execution. D. Y. and Z. W. participated in data analysis and interpretation. Z. W., Y. W., and H. C. performed animals and biochemistry experiments. Z. W., and Y. L., X. W., performed molecular biology and cell biology experiments. D. Y. wrote the manuscript. All of the authors have seen and commented on the manuscript.

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Ethics approval and consent to participate

All experimental protocols involving animals were approved by the Animal Care Committee of Shandong University.

Availability of data and materials

Not applicable.

Competing interests

We have no competing interests.

Consent for publication

Not applicable.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

Not applicable.

Research involving Human Participants and/or Animals

The research involving animals, all experimental protocols involving animals were approved by the Animal Care Committee of Shandong University.

Informed consent

Not applicable.

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Tables

Due to technical limitations, table PDF is only available as a download in the Supplemental Files section.

Figures

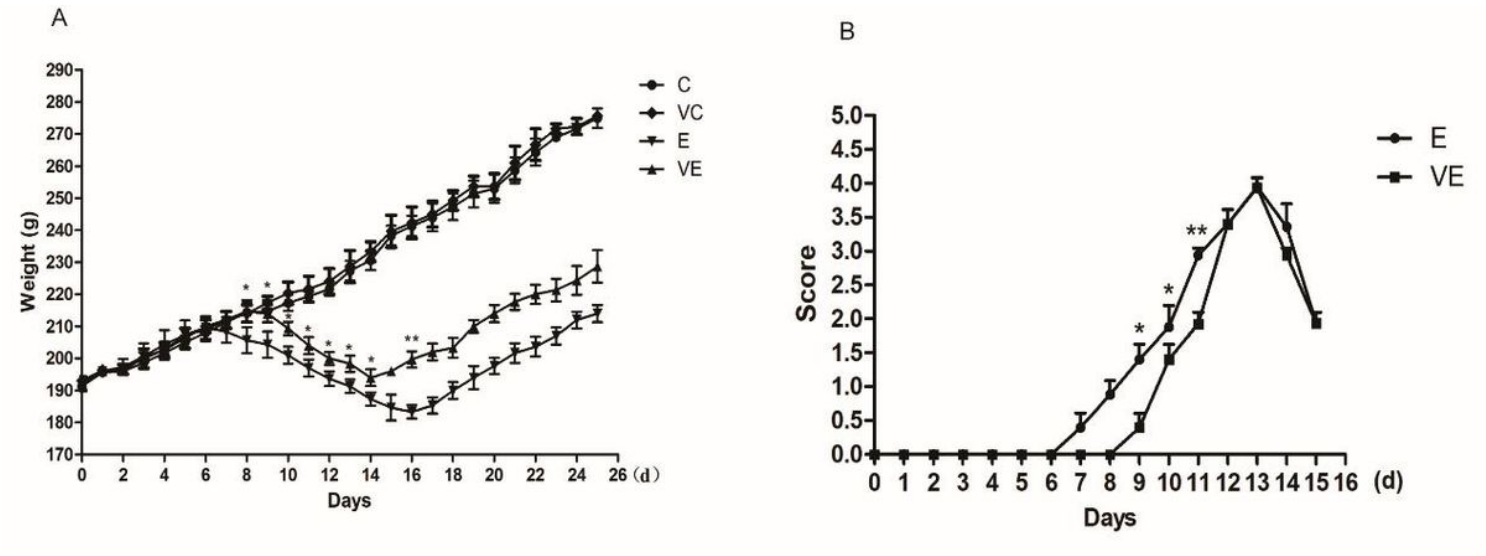

Figure 1

Vitamin D supplementation delays the onset of EAE rat model. A The weight change of rats from EAE (E and VE) and control(C and VC) after MBP injection. B Effects of vitamin D on the course of EAE disease progression as assessed by severity scoring after MBP injection (*p < 0.05, **p < 0.01).

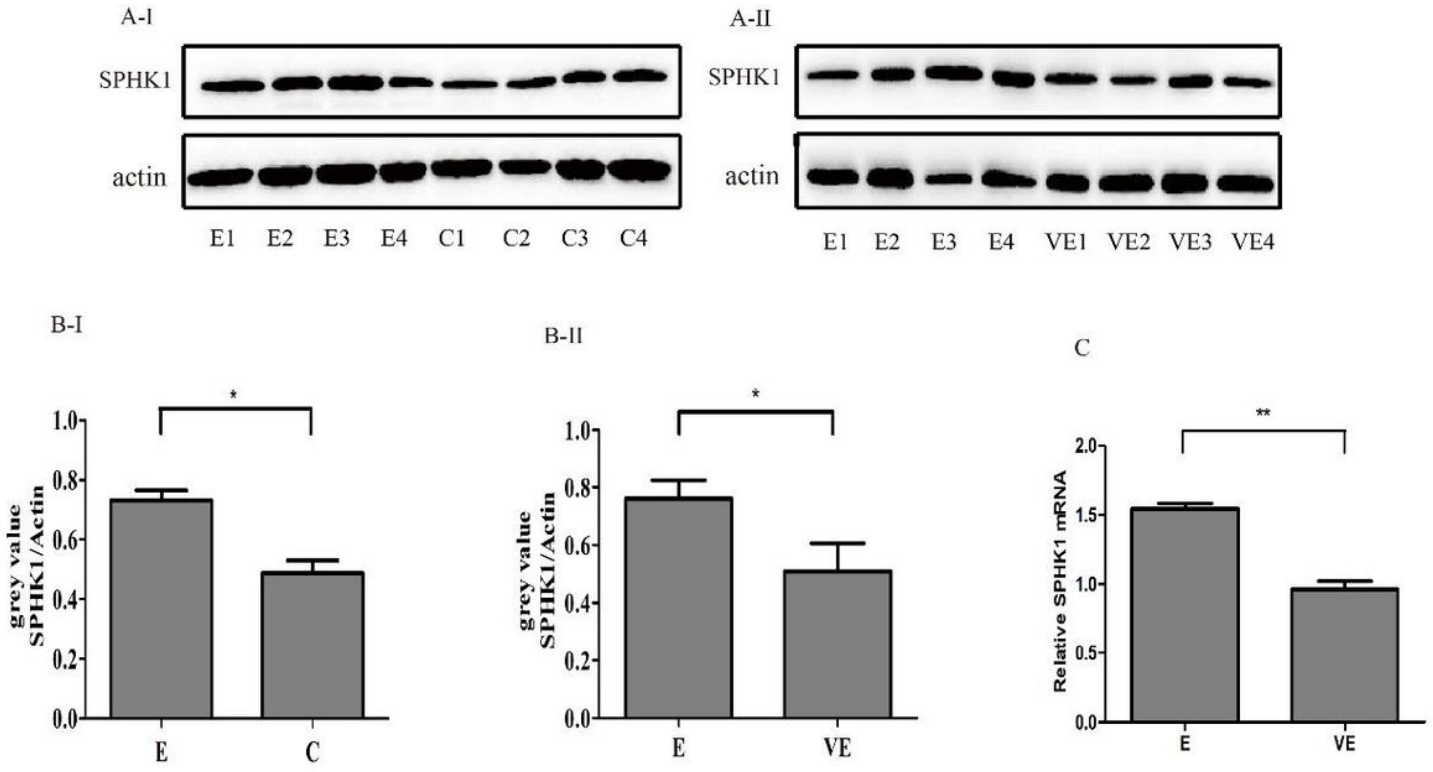

Figure 2

A-I Western blot analysis of SPHK1 levels in the spinal cord of EAE rats (E1–E4) or control rats (C1–C4). B-I The gray value SPHK1/Actin for each band associated with EAE group and control group was calculated with ImageJ software (*p < 0.05). A-II Western blot analysis of SPHK1 in the spinal cord of EAE rats with (VE1-VE4) and without (E1–E4) Vitamin D supplementation. B-II The gray value SPHK1/Actin for each band associated with EAE group and EAE with Vitamin D group was calculated with ImageJ software (*p < 0.05). C Real-time PCR of SPHK1 mRNA levels in the spinal cords of rats from each group. Results (mean + SD, performed in triplicates) are representative of three independent experiments (*p < 0.05).

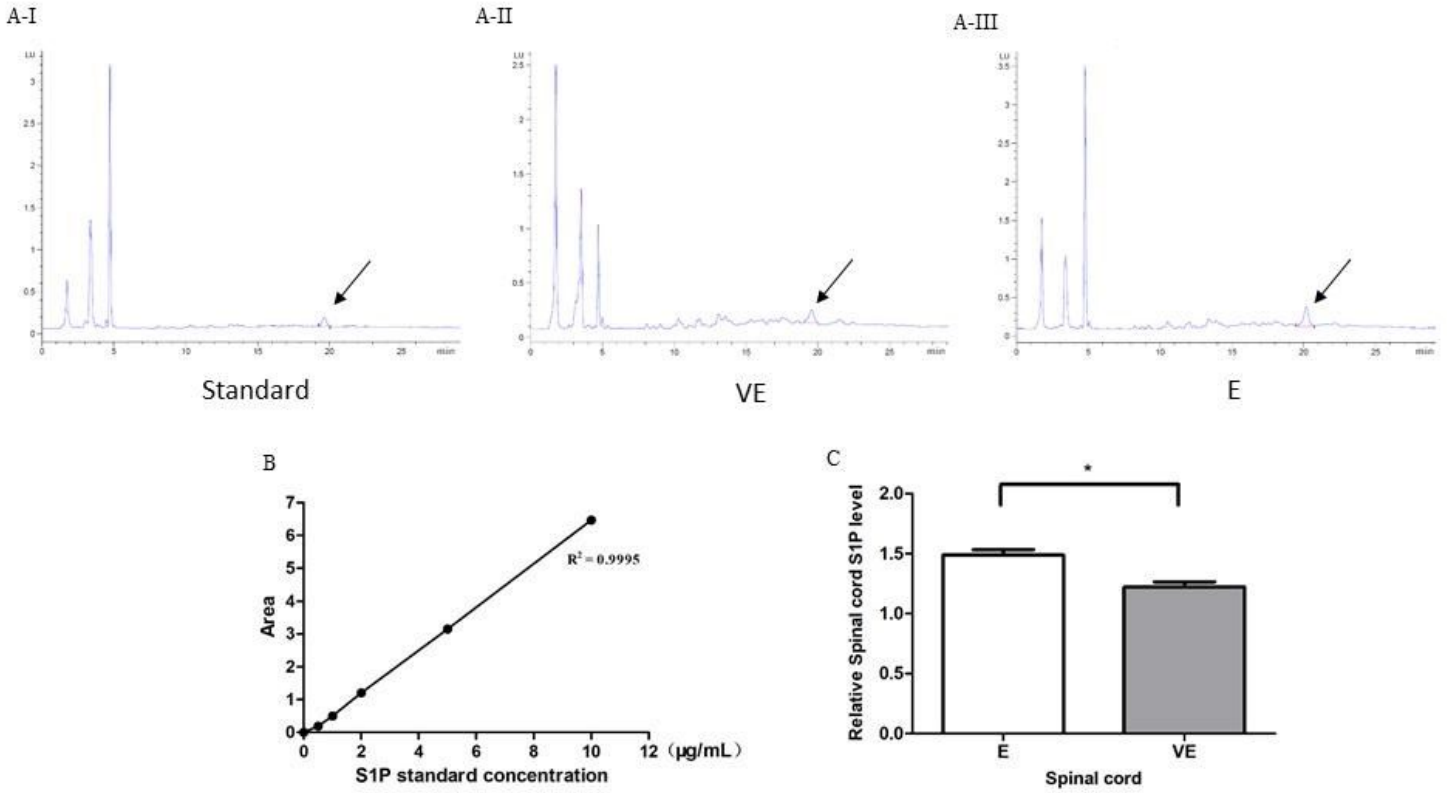

Figure 3

A HPLC spectrogram of S1P standard (A- I), S1P in the spinal cord of VE group rats (A- II), and S1P in the spinal cord of E group rats (A- III). B The standard curve of S1P. C The concentration of S1P in the spinal cords of rats was determined by HPLC (E and VE groups). (*p < 0.05).

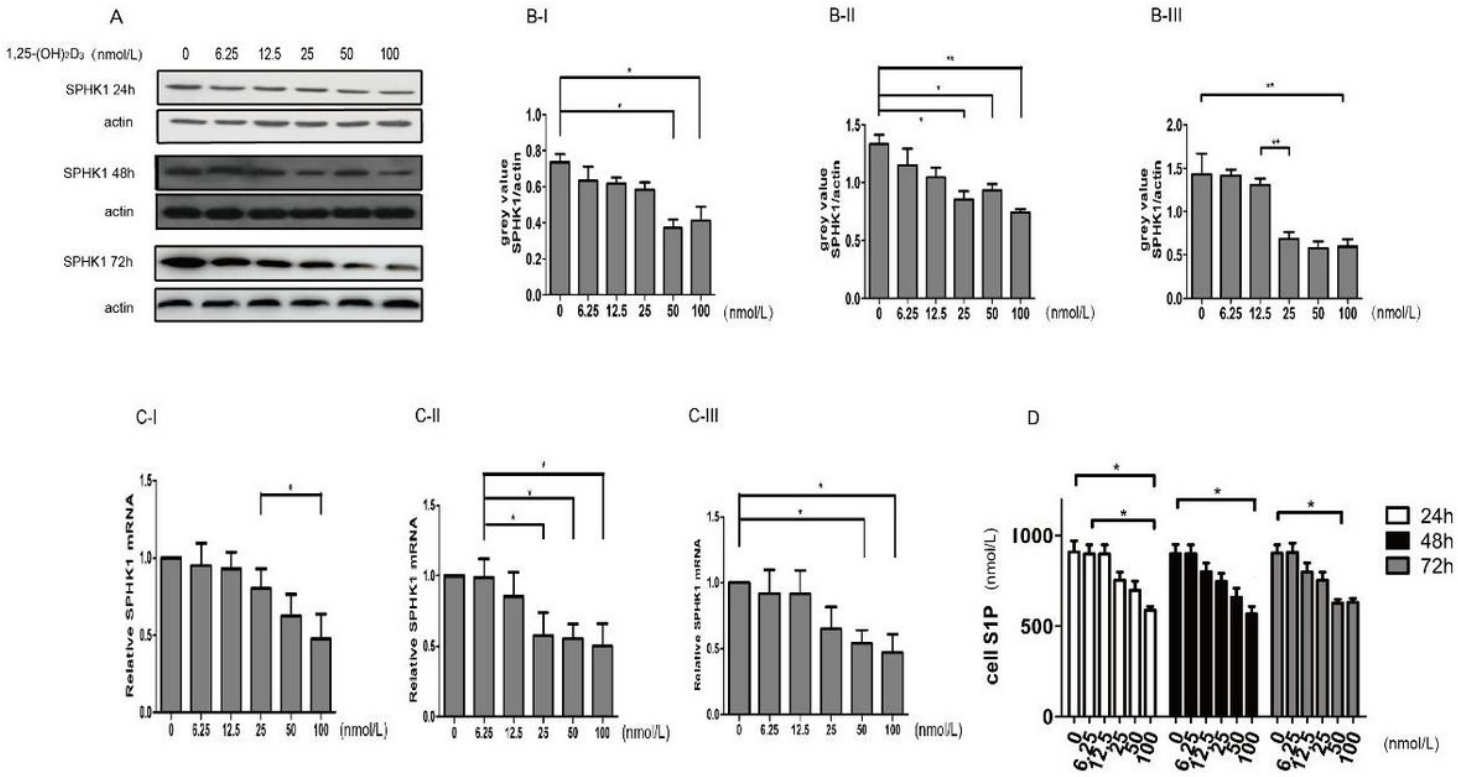

Figure 4

A Western blot analysis of SPHK1 levels in the PC12 cells stimulated with different concentration of 1,25-(OH)2D3 for 24 h, 48 h and 72 h. B The gray value for each band generated after 24 h(B-I) , 48 h(B-II) and 72 h(B-III)of stimulation was calculated with ImageJ software (*p < 0.05, **p < 0.01). C Real-time PCR of mRNA levels in PC12 cells stimulated with different concentrations of 1,25-(OH)2D3 for 24 h(C-I) , 48 h(C-II) and 72 h(C-III). Results (mean + SD, performed in triplicate) are representative of three independent experiments (*p< 0.05). D The concentration of S1P in PC12 cells stimulated with different concentrations of 1,25-(OH)2D3 for 24 h, 48 h and 72 h was measured by ELISA (*p < 0.05).

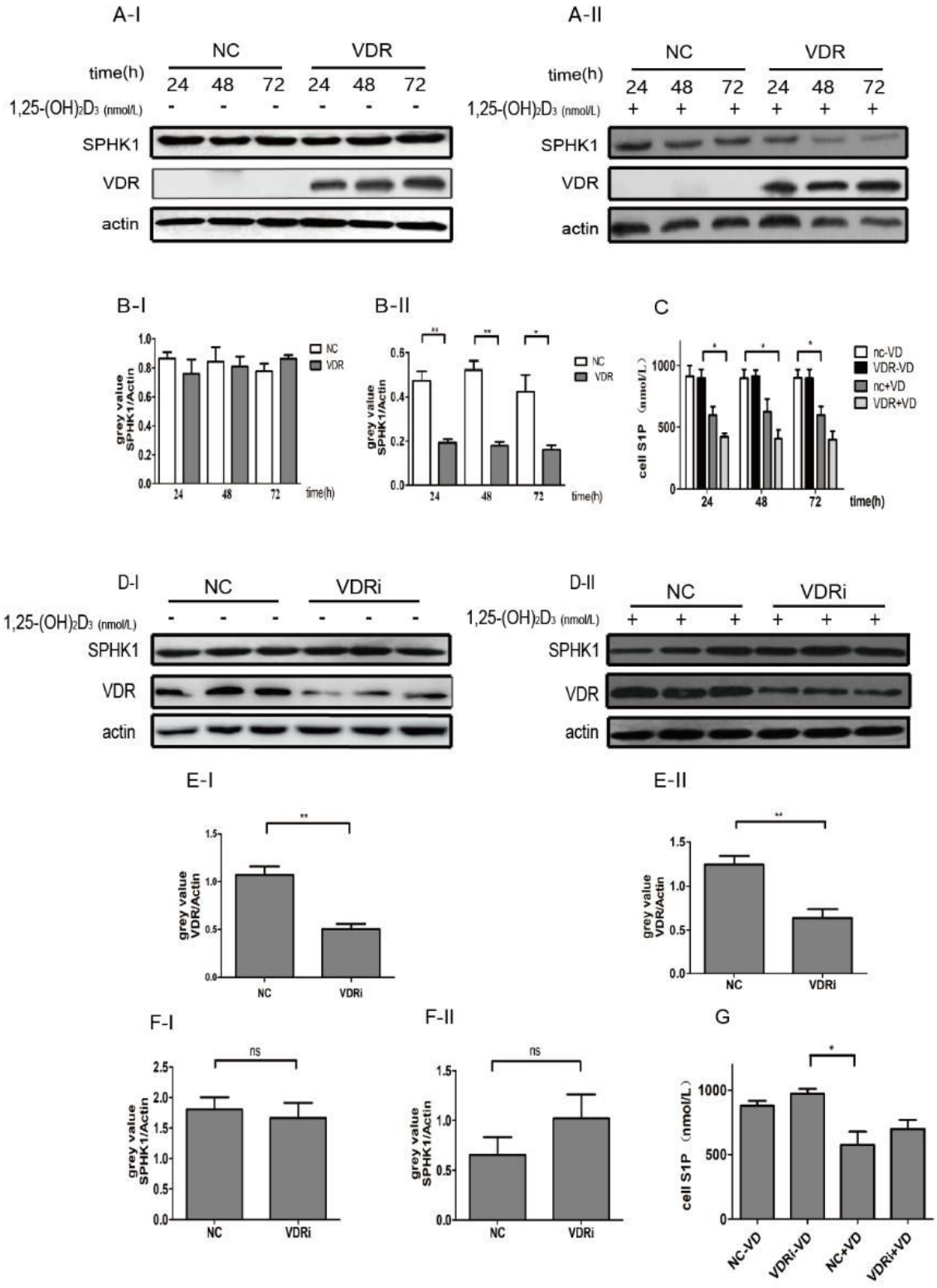

Figure 5

A PC12 Cells stimulated for 24, 48, or 72 h without(A-I) 1,25-(OH)2D3 (100 nmol/L) following transfection with the VDR plasmid (GV230) or control (NC) plasmid and PC12 cells stimulated for 24, 48, or 72 h with(A-II) 1,25-(OH)2D3 (100 nmol/L) following transfection with the VDR plasmid (GV230) or control (NC) plasmid. B-I The gray value for each band (following stimulation without 1,25-(OH)2D3) was calculated using ImageJ software. B-II The gray value for each band (following stimulation with 1,25-

(OH)2D3) was calculated using ImageJ software, *p < 0.05, **p < 0.01. C Concentration of S1P in PC12 cells. Cells were transfected with the VDR plasmid or control (NC) plasmid. The concentration of S1P in PC12 cells stimulated with or without 1,25-(OH)2D3 was measured by ELISA (*p < 0.05). D VDR interference in PC12 cells was verified by Western Blot analysis following injection of the Lentiviral expression vector and control Lentiviral expression vector. PC12 cells stimulated without(D-I) or with(D-II) 1,25-(OH)2D3 (100 nmol/L) following injection of the Lentiviral expression vector and control Lentiviral expression vector. E The gray value VDR/Actin for each band was calculated using ImageJ software(following stimulation without(E-I) or with(E-II) 1,25-(OH)2D3(**p < 0.01). F-I The gray value SPHK1/Actin for each band was calculated with ImageJ software (following stimulation without 1,25-(OH)2D3. F-II The gray value SPHK1/Actin for each band was calculated using ImageJ software (following stimulation with 1,25-(OH)2D3. G PC12 cells injected with the Lentiviral expression vector and control Lentiviral expression vector. The concentration of S1P in PC12 cells stimulated with or without 1,25-(OH)2D3 was measured using ELISA (*p < 0.05).

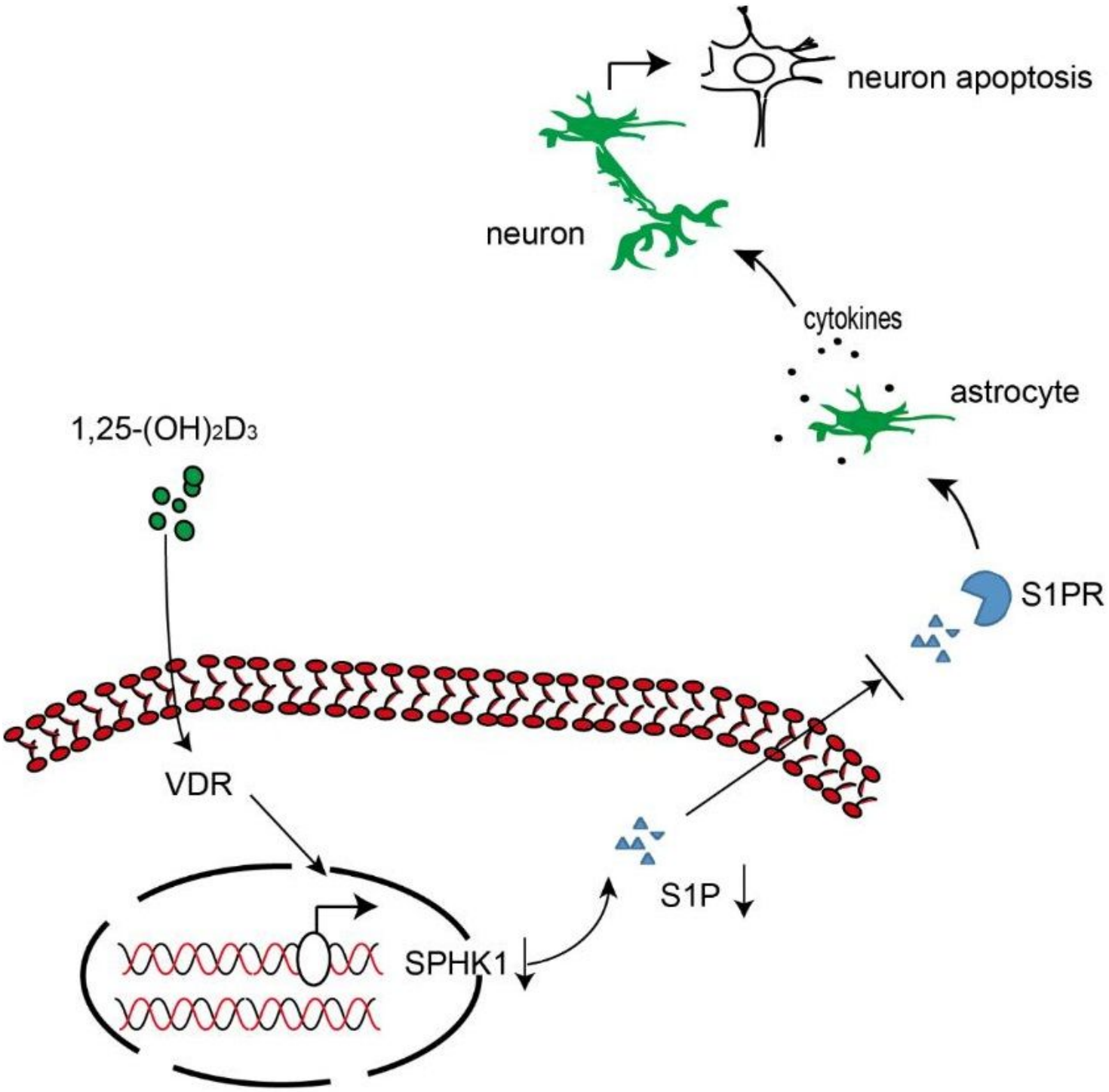

Figure 6

Model supported by this study. S1P binding to its receptor induces astrocytes to release cytokines. This interaction triggers demyelination and neuronal apoptosis. Vitamin D interaction with VDR reduces SPHK1 synthesis, which results from a reduction in S1P synthesis. which has favorable effects during the induction of EAE.

Supplementary Files

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