1,25-dihydroxyvitamin D3 attenuates NPSLE in MRL/lpr mice through a meliorating BCSFB activating PPARγ/NF-κB/TNF-α and lessening TGF-β 1/Smads

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Research Article

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Abstract

Backgrounds: Systemic lupus erythematosus encephalopathy (NPSLE) is a serious complication of systemic lupus erythematosus (SLE) involving the nervous system with high morbidity and mortality. A key hypothesis in NPSLE is that a disrupted barrier allowed autoantibodies and immune components of peripheral blood to penetrate into the central nervous system (CNS), resulting in inflammation and damage. The blood cerebrospinal fluid barrier (BCSFB), which consists of the choroid plexus and the hypothalamic tanycytes, has long been regarded as an immunological sanctuary site. 1,25-dihydroxyvitamin D3 (1,25-$(OH)_2D_3$) is the active form of vitamin D, which plays multiple roles in inflammation and immunoregulation. In this study, we investigate the possible protective effects of 1,25-dihydroxyvitamin D3 on BCSFB dysfunction of NPSLE in MRL/lpr mice and explore the mechanism of 1,25-dihydroxyvitamin D3 inhibiting the progression of NPSLE.

Methods: 40 MRL/lpr mice at 11-week-old were included in this study and the mice were divided into VitD3-treated group and control group. Mice in the VitD3-treated groups received 4μg/kg 1,25-dihydroxyvitamin D3 in 1% dimethyl sulfoxide (DMSO) intraperitoneal injection twice a week for 3 weeks. Mice in the control groups received 1% DMSO intraperitoneal injections for 3 weeks. The mice were anesthetized at 0 weeks (T1), 2 weeks (T2), 4 weeks (T3), and 6 weeks (T4) after treatment, and the mice were executed to collect blood and brain tissue samples. During this period, the skin lesions and neuropsychiatric manifestations of mice were observed continuously. The expressions of serum anti-double-stranded DNA (dsDNA) antibody and C3 complement were analyzed by ELISA. Then we evaluated the effect of 1,25-dihydroxyvitamin D3 on BCSFB integrity with time in terms of histopathological changes, neurological deficit, and the expression of brain-derived neurotrophic factor (BDNF) in MRL/lpr mice. BCSFB permeability and the expression of permeability-related proteins with time in the brain were also analyzed by immunofluorescence and western blotting respectively. To determine the possible mechanism underlying the role of 1,25-dihydroxyvitamin D3 in BCSFB maintenance, the effects of peroxisome proliferator-activated receptor-gamma (PPARγ) on BCSFB integrity and the expression of TGF-β/Smads on BCSFB integrity with time were detected.

Results: The skin lesions, neuropsychiatric manifestations, and pathology of mice in the VitD3-treated group were significantly improved compared with those in the control group. The levels of A-dsDNA in the VitD3-treated group were significantly lower than those in the control group ($P<0.05$). The C3 in the VitD3-treated group was significantly higher than those in the control group ($P<0.05$). The expressions of occluding and claudin-2 in the VitD3-treated group were significantly higher than those in the control group ($P<0.05$). 1,25-dihydroxyvitamin D3 treatment modulated the PPARγ/TNF-α/NF-κB axis and increased BDNF expression levels ($P<0.05$). There were significant differences in TGF-β/Smads pathway expression between the two groups ($P<0.05$). The TGF-β1, TβR-1, Smad2/3, and P-Smad2/3 in the VitD3-treated group were significantly lower than those in the control group ($P<0.05$). Furthermore, these results presented the descending or increasing tendency in the VitD3-treated group or the control group.

Conclusion: Our findings support the positive effect of 1,25-dihydroxyvitamin D3 on NPSLE in MRL/lpr mice, which may be related to the protection of BCSFB disruption through the activation of the anti-inflammatory PPARγ/NF-κB/TNF-α pathway as well as upregulation of BDNF and the inhibition of TGF-β/Smads signaling pathway.

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with multiple pathogenic factors and mechanisms, involving multiple organs and systems (1). Central nervous system (CNS) involvement in lupus and neuropsychiatric lupus (NPSLE) are serious complications of SLE, which increase the morbidity and mortality rates significantly (2). Patients with NPSLE can demonstrate a considerable variety of brain injuries with neurological and psychiatric features, including cognitive impairment and mood disorders (3). Due to the complexity of pathophysiologic mechanisms involved and limited access to tissue, its pathogenesis has not yet been well characterized (2). Many factors may be involved in the pathogenesis of NPSLE. On the one hand, it may be related to autoimmune activation, which may have an impact on brain function through chronic inflammatory reactions or proinflammatory neuropeptides (4). On the other hand, it may be related to the entry of circulating neuropathic antibodies to the brain via a pathologically permeable barrier. Thus, these affect the emotion or cognition of MRL/lpr mice eventually (5).

1,25-dihydroxyvitamin D3 is the active form of vitamin D, which plays a crucial role in calcium and phosphorus metabolism (6). In recent years, the immunomodulatory role of 1,25-dihydroxyvitamin D3 in inhibiting the inflammatory response and protecting neurons have attracted much attention (7). It has been reported that the positive rate of Anti-double-stranded DNA (A-dsDNA) decreased significantly in SLE patients with vitamin D as a supplementary therapy (8). Therefore, it is of great significance to find safe and effective treatment and explore the possible therapeutic mechanism.

A-dsDNA antibody plays an important part in organ injuries of SLE by indirect and direct binding with antigens (9). In addition, the degree of reduction in serum levels of C3, which is composed in the complement pathway, is associated with the occurrence, development, and prognosis of SLE (10).

Nevertheless, abnormal antibody brain penetrance might actually result from a dysfunction in any of the three brain barriers: the blood-brain barrier (BBB), the meningeal barrier, or the blood-cerebrospinal fluid barrier (BCSFB) (11). Newer studies demonstrate that the BBB damage may not always be required as previously believed (4). Recently, there has been a study supported that the BCSFB barrier dysfunction, which is a causative factor in the disease rather than BBB, facilitates the brain’s exposure to self-antibodies and that abnormal lymphocytes can enter cerebral ventricles in NPSLE (12). Although both barriers have similar functions, they differ in their morphologic, transport mechanisms, and functional properties, resulting in different degrees of selectivity (11). Here, we examined BCSFB integrity in MRL/lpr, an established NPSLE mouse model.

And the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPARγ) is a ligand-activated transcription factor that plays several pivotal roles in the control of gene expression linked to a variety of physiological processes, including the regulation of inflammatory mediators and immunity responses (13). NF-κB and its downstream inflammatory response signal activation are regulated by the PPARγ that is the best-known PPAR isoform, has shown the protective effect of brain injury and neurons (14). There is a study has shown that the activation of PPAR-γ reduces NF-κB and its downstream proinflammatory cytokines, which decreases brain inflammation and protects neuronal by maintaining blood-brain barrier function in the brain (15). Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophic factor in the central nervous system where it plays a key role in synaptic plasticity and neuronal survival (16). The activation of PPAR-γ is also known to upregulate BDNF (15). However, the effects of PPARγ on BCSFB integrity and its function are largely unknown in MRL/lpr.

Moreover, it has been confirmed that the TGF-β family regulates the diversity of cellular processes, including proliferation, differentiation, apoptosis, and migration (17). In general, TGF-β mediates its biological activity through Smads signaling pathway (18). Inflammatory responses and autoimmune activations are the key factors in the pathogenesis of NPSLE (3). And TGF-β plays a key role in the CNS as a response to injury and functions as a signal (19). Thus, we supposed that the overexpression of the inflammatory factor TGF□β may participate in the process of NPSLE. In addition, the Smads signaling pathway is considered to mediate TGF-β and plays an important role in the development of NPSLE. Given the above, the signaling pathway may be associated with the processes of NPSLE, which may be an important target to NPSLE. However, the effects of the TGF-β/Smads signaling pathway on BCSFB integrity and its function remain unclear so far in MRL/lpr.

Therefore, in this study, we focus on the regulative effect of 1,25-dihydroxyvitamin D3 on NPSLE and explain how it reduced cerebral injury. We hypothesized that 1,25-dihydroxyvitamin D3 maintains BCSFB integrity by upregulating BDNF levels, possibly via the PPARγ-dependent anti-inflammatory pathway and TGF-β/Smads signaling pathway. Our findings show that 1,25-dihydroxyvitamin D3 plays an important role in ameliorating NPSLE by preserving the BCSFB via PPAR-γ activation and TGF-β/Smads inhibition.

Materials And Methods

Mice and treatment

Forty female MRL/lpr mice at 8-week-old (25.0±3.5g) were obtained from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China) and the quality certificate number: SCXK hu 2017-0005. All experimental protocols used in this study were approved by the Institution Ethical Committee of Weifang Medical University. Mice were housed under specific pathogen-free (SPF) conditions with temperature (22±3°C)□humidity (52.5±2.5%) and illumination for 12h/day. The animals were allowed free access to standard diet and tap water and acclimatized to the environment for two weeks before the experiments.

40 MRL/lpr mice at 11-week-old were included in this study and the mice were divided into VitD3-treated group and control group by random number table method，20 mice in each group. Mice in the VitD3-treated groups received 4μg/kg 1,25-dihydroxyvitamin D3 （Sigma-Aldrich Co., St. Louis, MO, USA) in 1%dimethyl sulfoxide（DMSO, Sigma-Aldrich Co., St. Louis, MO, USA）intraperitoneal injection twice a week for 3 weeks. Mice in the control groups received 1% DMSO intraperitoneal injections for 3 weeks. The mice were anesthetized with 1% Pentobarbital (35 ml/kg i.p.) at 0 weeks (T1), 2 weeks (T2), 4 weeks (T3), and 6 weeks (T4) after treatment, and the mice were executed to collect blood and brain tissue samples (Fig. 1). During this period, the skin lesions and neuropsychiatric manifestations of mice were observed continuously. The expressions of serum A-dsDNA antibody and complement C3 were analyzed by ELISA. The pathological changes were observed by HE and Nissl staining. The BCSFB was analyzed by western blot and immunofluorescence. Furthermore, the effects of PPARγ on BCSFB and its function were detected by western blot and ELISA. The expression of TGF-β/Smads was detected by western blot and RT-PCR.

Histopathological analysis

The brain tissue samples were fixed with 4% paraformaldehyde. After deparaffinization and rehydration, the 5μm paraffin sections were used for Hematoxylin and eosin (HE) and Nissl stains.

Immunofluorescence

5μm paraffin sections were washed with phosphate-buffered saline (PBS) and incubated with blocking solution. Slides were incubated with primary antibodies occluding (1:1000, Abcam, Inc.UK) at 4°C overnight. Slides were then washed with PBS, incubated with secondary antibodies, and mounted. All the images were obtained through a microscope equipped with a color camera (Nikon Eclipse Ni, Japan).

Enzyme linked immunosorbent assay (ELISA) analysis

After the mice were anesthetized with pentobarbital, the blood was collected by eyeball extraction. Carefully collect the serum samples after centrifugation (4℃, 4000r, 10min), and detect the serum A-dsDNA、C3、NF-κB and TNF-α levels by ELISA kit (Wuhan Boster Bio-engineering Co. Ltd., Wuhan, China). The optical density values were read at 450nm.

Western blotting analysis

Total protein was extracted from brain tissue samples with RIPA lysing buffer (Thermo Fischer Scientific, Inc. Waltham, MA), following the manufacturer's protocols. After quantification by BCA kit, denaturation, and electrophoresis, it was transferred to PVDF membranes (Millipore, Bedford, MA, U.S.). The membranes were incubated with occluding (1:1000, Abcam, Inc.UK), claudin-2(1:1000, Invitrogen AB, Lidingö, Sweden). PPARγ (1:1000, Abcam, Inc.UK), NF-κB (1:1000, Abcam, Inc.UK), TNF-α (1:1000, Abcam, Inc.UK), BDNF (1:1000, Abcam, Inc.UK), TGF-β1 (1:1000, Abcam, Inc.UK), TβR-I (1:1000, Abcam, Inc.UK), Smad2/3 (1:1000, Cell Signaling Technology Inc. USA), P-Smad2/3 (1:1000, Abcam, Inc.UK) and GAPDH (1:10,000, Abcam, Inc.UK) at 4 °C overnight. Then the membranes were incubated with the horseradish

peroxidase-conjugated secondary antibody for 2h. The target protein was detected and analyzed with ImageJ software (National Institutes of, Bethesda, MD, USA).

Real-time quantitative PCR analysis

Total RNA was extracted from brain tissue samples by Trizol reagent (Takara Biotechnology, Dalian, China), according to the manufacturer's directions. The cDNA was generated by using a reverse transcription-polymerase chain reaction (RT-PCR) kit (Takara Biotechnology, Dalian, China). RT-PCR was carried out for TGF-β1, TBR-1, Smad2/3, and GAPDH using LightCycler480 II (Roche). The levels of genes expression were analyzed by the equation $2^{-(\Delta\Delta Ct)}$. PCR primer sequences were listed in Table 1.

Table1. Primer sequences were used in the study. (Primer Premier 5.0)

Genes	Forward primers (5'–3')	Reverse primers (5'–3')
TGF-β1	AACAATTCCTGGCGTTACCTT	GCCCTGTATTCCGTCTCCTT
Smad2	GTGTTTGCTGAGTGCCTAAGTG	ACAGACTGAGCCAGAAGAGCA
Smad3	AGACATTCCACGCCTCACAG	AGGCACTCCGCAAAGACC
GAPDH	GGTTGTCTCCTGCGACTTCA	TGGTCCAGGGTTTCTTACTCC

Statistical analysis

The statistical analysis was performed with SPSS version 20.0 software. Results were presented as mean ± standard deviation and analyzed by t-test or one-way ANOVA. A p value less than 0.05 was considered to be statistically significant.

Results

1,25-dihydroxyvitamin D3 improved skin lesions and neuropsychiatric manifestations

The MRL/lpr mice began to have different degrees of skin lesions at the age of 8 weeks, especially in the skin of the head, back, mouth, and nose (black arrow). At 20-week-old, the skin lesions of mice in the treated group were significantly improved than those in the control group (Fig. 2). The MRL/lpr mice showed obvious memory deficits and depression at the age of 8 weeks, which was characterized by typical systemic lupus erythematosus encephalopathy, such as lack of curiosity, indifference to external stimuli, liking to be alone, and lack of activity. However, the mice in the treated group improved evidently compared with those in the control group. Mice increased activity and responded to new things positively.

1,25-dihydroxyvitamin D3 ameliorated cerebral injury

Choroid plexus in brain tissue was observed by HE staining（Fig.3A）. The brain tissue in the control groups showed lymphocytic infiltrate (black arrow) with hemorrhage (green arrow) in the choroid plexus.

Moreover, the hippocampus was observed by HE (Fig.3B) and Nissl's (Fig.3C) staining. Neuronal shrinkage, deeper stained of cells, the unclear boundary between nucleus and cytoplasm (yellow arrow) can be seen in the hippocampus. And neuronal degeneration and cytoplasmic vacuolation were obvious in the hippocampus (red arrow). The findings demonstrated that MRL/lpr mice had brain damage. In contrast, 1,25-dihydroxyvitamin D3 treatment significantly reduced the pathological damage in the brain via decreasing inflammatory injury and protecting neurons. Taken together, these findings indicated that 1,25-dihydroxyvitamin D3 could ameliorate cerebral injury. The pathological changes at the different time points of the brain were observed by staining, which indicated that with the increase of time, the pathological changes became worse.

1,25-dihydroxyvitamin D3 reduced A-dsDNA and increased C3

Anti-dsDNA is an SLE specific autoantibody, which is related to SLE activity closely. The expression of serum A-dsDNA in the control group increased with time ($P<0.05$). The treatment of 1,25-dihydroxyvitamin D3 reduced the A-dsDNA notably but there was no significant difference between the treated groups ($P<0.05$). In addition, complement C3 plays an important role in the pathway of complement activation and it's also associated with SLE activity. In the treated group, 1,25-dihydroxyvitamin D3 increased the level of complement C3 significantly compared to the mice in the control group. However, compared with the mice in the T1 control group or treated group, the level of complement C3 in the T2, T3, and T4 control group or treated group decreased correspondingly ($P<0.05$), which may be related to the activity of SLE. (Table2、Fig4)

Table2. The expression of serum ds-DNA antibody and C3 complement in MRL/lpr mice

Group	n	A-ds-DNA(ng/ml)				C3(μg/ml)			
		T1	T2	T3	T4	T1	T2	T3	T4
Control	20	20.65 ±0.79	24.51 ±0.59	26.05 ±0.88	28.99 ±2.70	93.26 ±6.84	75.12 ±5.45	71.14 ±1.97	57.93 ±1.89
Treated	20	14.94 ±0.86	15.29 ±1.78	16.98 ±0.89	17.46 ±0.78	129.27 ±11.74	104.81 ±5.21	97.79 ±7.88	104.27 ±6.73

1,25-dihydroxyvitamin D3 protected the BCSFB.

To evaluate whether 1,25-dihydroxyvitamin D3 also protected the BCSFB, brain sections were stained by immunofluorescence assay, and the expression of permeability-related proteins with time in the brain was also analyzed by western-blot. As shown in Fig.5A, except T1, the expressions of occludin and claudin-2 in the VitD3-treated group were significantly higher than those in the control group (P 0.05), whereas increased with time. At the time of T1, the result explained 1,25-dihydroxyvitamin D3 may have

not quick efficacy on BCSFB or the sample size may be insufficient. Then we found significantly increased occludin of VitD3-treated mice by immunofluorescence (Fig.6). These results indicated that 1,25-dihydroxyvitamin D3 protected MRL/lpr mice from cerebral injury on BCSFB permeability and integrity. However, the cerebral injury on BCSFB got worse with time.

1,25-dihydroxyvitamin D3 suppressed the inflammatory responses by activating PPARγ

Since inflammation is the major pathogenic basis of NPSLE, we evaluated the effects of 1,25-dihydroxyvitamin D3 on the levels of inflammatory mediators. 1,25-dihydroxyvitamin D3 treatment markedly reduced the levels of the proinflammatory transcription factor NF-κB and proinflammatory cytokine TNF-α in the MRL/lpr, whereas increased with time. In addition, 1,25-dihydroxyvitamin D3 treatment increased the expression of BDNF that decreased with time (Fig. 7). Therefore, 1,25-dihydroxyvitamin D3 treatment suppressed the inflammatory response on the MRL/lpr by modulating the PPARγ/NF-κB/TNF-α axis and increasing BDNF expression levels.

1,25-dihydroxyvitamin D3 inhibited the TGF-β1/Smads signaling pathway

To explain the protective mechanism of 1,25-dihydroxyvitamin D3 on NPSLE, the activation of the TGF-β/Smads pathway was detected. PCR assay showed that, compared with the control group, the treatment groups significantly decreased the mRNA expression levels of TGF-β1, Smad2, and Smad3, whereas increased with time. (Fig. 8A). Additionally, the protein expression levels of TGF-β1, TβR-I, Smad2/3, and P-Smad2/3 in the control group were higher than those of the treated group. 1,25-dihydroxyvitamin D3 treatment significantly decreased the expression levels of TGF-β1, TβR-I, Smad2/3, and P-Smad2/3 compared with the control group, while increased those proteins expressions with time (Fig. 8B). These results suggested that 1,25-dihydroxyvitamin D3 could suppress the activation of the TGF-β/Smads pathway to ameliorate systemic lupus erythematosus encephalopathy.

Discussion

In this study, we explored the effect of 1,25-dihydroxyvitamin D3 on systemic lupus erythematosus encephalopathy using MRL/lpr mice which have proven to be the most useful spontaneous model of NPSLE. Our results showed that 1,25-dihydroxyvitamin D3 was able to protect MRL/lpr mice from cerebral injury by presenting serum immunological markers and pathological changes. However, the pathogenesis of NPSLE is highly complex, with detailed mechanisms yet to be elucidated. In order to investigate the underlying mechanism of 1,25-dihydroxyvitamin D3 against NPSLE, the BCSFB integrity, the effects of PPARγ/NF-κB/TNF-α and TGF-β/Smads on BCSFB were detected. Our findings support the positive effect of 1,25-dihydroxyvitamin D3 on NPSLE in MRL/lpr mice, which may be related to the protection of BCSFB disruption through the activation of the anti-inflammatory PPARγ/NF-κB/TNF-α pathway and upregulation of BDNF and the inhibition of TGF-β/Smads signaling pathway expression.

However, definitive biomarkers of NPSLE are yet to be identified owing to its complex pathogenesis and polymorphic phenotype (20). And we analyzed the identification of pertinent, robust biomarkers of SLE.

The MRL/lpr mice have a very similar overall disease pattern to human SLE including cutaneous manifestation (21). To expose the effect of 1,25-dihydroxyvitamin D3 on SLE, we firstly observed cutaneous manifestations in MRL/lpr mice. Skin lesions are one of the typical symptoms of SLE. The MRL/lpr mice began to have different degrees of skin lesions at the age of 8 weeks, especially in the skin of the head, back, mouth and nose. However, 1,25-dihydroxyvitamin D3 treatment significantly attenuated the degree of skin lesions. The immunopathogenic mechanisms underlying the development of end-organ disease in SLE have been made much progress (1). These involve complex immunological cascades, including humoral mediated immunity such as autoantibody and complement deposition. Autoantibodies to components of chromatin, which include dsDNA, are central in the pathogenesis of SLE (9). The activation of the complement system in SLE is characterized by the consumption of complement proteins. The degree of reduction in serum level of C3, which is composed in the complement pathway, is associated with the pathogenesis of SLE (22). Serum A-dsDNA and C3 activities showed markedly increased or decreased with time after onset, which may be associated with lupus activity. And it may cause subsequent leukocyte and cytokine driven serious multi-system damage including the nervous system; while 1,25-dihydroxyvitamin D3 treatment notably improved the activity of these serum markers. These findings demonstrate that 1,25-dihydroxyvitamin D3 has a protective effect against SLE and NPSLE, to some extent.

The symptoms of NPSLE in MRL/lpr mice are caused by autoimmune diseases in most cases (23). To further investigate the protective effect of 25-dihydroxyvitamin D3 on NPSLE, we observed neuropsychiatric manifestations in MRL/lpr mice. Neuropsychiatric manifestations are the most common diffuse clinical manifestation of the central nervous system in NPSLE. Neuropsychiatric impairment depends on the environment, genetic inheritance, and hormonal factors. Headaches, mood disorders, cognitive dysfunction, seizures, and cerebrovascular disease are the most frequent symptoms (24). Vascular abnormalities, self-autoantibodies, and inflammatory mediators are considered to be the primary contributory factors (25). In the present study, 1,25-dihydroxyvitamin D3 treatment improved notably the typical neurological symptoms of NPSLE, such as lack of curiosity, indifference to external stimuli, liking to be alone, and lack of activity. In addition, the HE and Nissl staining showed severe histological damages to brain tissue. Obviously, the nerve cells in the brain of MRL/lpr mice were extensively damaged, especially in the hippocampus, showing neuronal atrophy and nuclear chromatin condensation. Neuronal damage in the hippocampus may trigger NPSLE development (26). At the same time, previous studies showed that severe gene levels in the hippocampus were related to neuropsychiatric manifestations, including cognitive function (27). Similar damages of the immune cells were also observed in the choroid plexus of MRL/lpr mice. As shown in HE staining, lymphocytic infiltrated with hemorrhage in choroid plexus of MRL/lpr mice. It may be due to the influx of immune cells, self-antibodies, pro-inflammatory cytokines that occur after barrier disruption in the brain. The findings demonstrated that MRL/lpr lupus mice had brain damage. However, 1,25-dihydroxyvitamin D3 treatment significantly reduced the degree of brain damage of NPSLE. These findings explain that 1,25-dihydroxyvitamin D3 can ameliorate brain damage of NPSLE in the MRL/lpr mice by protecting neurons and decreasing inflammatory injury.

The pathogenesis of NPSLE remains poorly understood and there remain many unanswered questions regarding its mechanism. However, it is believed that important factors are the immune cell-mediated damage and pathological activity that may result in disruption of barrier in the brain (28). When its integrity is compromised, immune cells, self-autoantibodies, and proinflammatory cytokines can cross barrier by promoting an inflammatory environment with glial activation, neurodegeneration, and subsequent adverse behavioral consequences (29,30). There are three brain barriers: the BBB, the meningeal barrier, and the BCSFB. It has been shown that BBB disruption can trigger NPSLE (31). However, in our study, the primary site of leukocyte entry into the brain in NPSLE likely occurs at the choroid plexus. And considerable intrathecal lymphocyte infiltration likely occurs through the BCSFB, accompanied by epithelial hyper-permeability to antibodies. Thus, we report that the pathogenesis of NPSLE is believed to include the entry of circulating neuropathic antibodies to the brain via a pathologically permeable BCSFB, pathogenesis aligned with the previous report of Sivan Gelb (32). Based on our findings, we focus on the BCSFB dysfunction as a causative factor in NPSLE. The choroid plexus is an epithelial bilayer surrounded by a highly vascularized capillary plexus (33). The epithelium synthesizes cerebrospinal fluid (CSF) but also separates brain ventricles from the blood to form BCSFB (34). The CSF space is separated from the vascular system via BCSFB, whereas BBB, responsible for maintaining the brain homeostasis, is located between the vascular system and the brain parenchyma (35). The apical tight junctions (TJs) of the choroid plexus (CP) epithelial cells play a fundamental role in the regulation of BCSFB permeability and integrity (33). The TJs are composed of proteins related to the inner and outer leaflets of the cell membrane, whereas occludin and claudins are the main transmembrane molecules mediating epithelial contact (36). Epithelial TJ of the BCSFB contains the protein claudin-2 (37). Thus, the expressions of occludin and claudin-2 purportedly reflect the integrity of BCSFB. In this study, we found that 1,25-dihydroxyvitamin D3 protected the BCSFB permeability and integrity in NPSLE of MRL/lpr mice. And the cerebral injury on BCSFB got worse with time. It was found that reduced expressions of occludin and claudin-2 following 1,25-dihydroxyvitamin D3 treatment is time-dependent.

PPARγ has been implicated in the pathogenesis of numerous diseases including diabetes, stroke, inflammatory or immune diseases. It is expressed in multiple cells, involving immune cells, and neurons (38). PPARγ not only exerts effects on the regulation of cellular differentiation to control lipid metabolism and glucose homeostasis (39). PPARγ ligands also play a crucial role in attenuating degenerative processes in central nervous systems as well as in peripheral systems (40). And it is responsible for the control of neurogenesis, anti-inflammatory mechanisms, and neuronal death (38,39,40). Previous studies have shown the gratifying anti-inflammatory effect of PPARγ, mainly via negatively regulating several transcription factors such as NF-κB and its downstream pro-inflammatory cytokine TNF-α (41). Therefore, regulating PPARγ and PPARγ-related pathways has great potential in treating NPSLE. In this study, exploration of the potential mechanisms demonstrated that 1,25-dihydroxyvitamin D3 significantly activated PPARγ and subsequently suppressed NF-κB and TNF-α activation. And in the serum of MRL/lpr mice, the levels of NF-κB and TNF-α markedly reduced in the VitD3-treated groups, suggesting that the result again, to some extent. Furthermore, the expression of the PPARγ/NF-κB/TNF-α axis changed over

time, as has been shown in the BCSFB. And there is a study has shown that the activation of the PPAR-γ/TNF-α/NF-κB axis decreases brain inflammation and protects neuronal by maintaining BBB function in the brain (15). Thus, it seems that the elevated expression of NF-κB along with upregulation of TNF-α, regulated by the PPARγ may induce alteration of BCSFB. And 1,25-dihydroxyvitamin D3 treatment protected BCSFB of NPSLE in the MRL/lpr mice by modulating the PPARγ/NF-κB/TNF-α axis.

BDNF, which has prominent functions of promoting neuroprotection and neurodegeneration, is one of the most widely extensively studied and distributed neurotrophins in the brain (42). Immune cell neurotrophin production could be neuroprotective against autoimmunity-driven CNS damage (43). However, there are many questions that whether BDNF is associated with damage severity in NPSLE. In this study, BDNF reduced with the damage severity in NPSLE, but 1,25-dihydroxyvitamin D3 treatment upregulated BDNF, which exerts a neuroprotective effect against brain injury of NPSLE.

To further investigate the underlying mechanism of 1,25-dihydroxyvitamin D3 on brain injury of NPSLE, the key TGF-β/Smads signaling pathway was detected. TGF-β1 is implicated in a large number of interactions and plays a variety of roles according to the cellular environment (44). Firstly, TGF-β is one of the most important cytokines to activate and promote the transformation, exacerbating the process of inflammation (45). And TGF-β is also associated with the immune dysregulation disorders such as Multiple Sclerosis (MS), in which a forceful increase in TGF-β expression and circulating TGF-β was observed during an MS attack in patients (46). Furthermore, TGF-β has been expressed in neural progenitor cells, differentiated neurons, and mature neural cells (19). In fact, TGF-β induces cell cycle exist the hippocampal neurons in mice (47) and is associated with the loss of adult neurogenesis by preventing the proliferation of progenitor cells (48). Previous studies of Alzheimer's and Parkinson's disease declared that TGF-β ligands were elevated in cerebrospinal fluid (49,50). At the same time, inflammatory response, neuronal damage, and autoimmune activation are the key factors in the pathogenesis of NPSLE (3). Smads are intracellular signal transduction molecules of the TGF-β superfamily. TβR-I and TβR-II-mediated TGF-β/Smads signal family members, and Smads were activated by TGF-β receptor phosphorylates downstream receptor. TGF-β causes phosphorylation of Smad2 and Smad3 and subsequently migrates to the nucleus, leading to the expression of target genes increased obviously (51). There was evidence to illustrate that the TGF-β/Smads signal pathway is related to NPSLE and 1,25-dihydroxyvitamin D3 treatment significantly down-regulated the expression of TGF-β1、TβR-I、Smad2/3 and phosphorylation of Smad2/3 in brain tissue, suggesting that the anti-inflammatory effect of 1,25-dihydroxyvitamin D3 may be due to the suppression of the TGF-β/Smads signaling pathway. However, the effect of the TGF-β/Smads on the BCSFB and its function in the brain remain unclear in MRL/lpr. Thus, in order to detect the effect of time points on the expression level of the TGF-β/Smads signaling pathway, the MRL/lpr mice were executed for 4-time points. In the present study, the expression of the TGF-β/Smads signaling pathway increased over time, as has been shown in the BCSFB. Based on these results, we inferred that the elevated expression of the TGF-β/Smads signaling pathway may induce alteration of BCSFB. And 1,25-dihydroxyvitamin D3 treatment protected BCSFB of NPSLE in the MRL/lpr mice by modulating TGF-β/Smads signaling pathway

Overall, this study demonstrates that the 1,25-dihydroxyvitamin D3 can ameliorate systemic lupus erythematosus encephalopathy in MRL/lpr mice, which may be related to the protection of BCSFB disruption through the activation of the anti-inflammatory PPARγ/NF-κB/TNF-α pathway as well as upregulation of BDNF and the inhibition of TGF-β/Smads signaling pathway. Moreover, it has few side effects or toxicity, suggesting that 1,25-dihydroxyvitamin D3 may be developed as a potential natural medicine for the treatment of systemic lupus erythematosus encephalopathy. Because of the limits of the experiments, this thesis is not included the inhibitor group but preliminary proves that the relationship between BCSFB and PPARγ/NF-κB/TNF-α pathway as well as TGF-β/Smads pathway, providing the foundation for successive study.

Abbreviations

NPSLE= Systemic lupus erythematosus encephalopathy; SLE=systemic lupus erythematosus; CNS=central nervous system; BCSFB= blood cerebrospinal fluid barrier; 1,25-(OH)2-VitD3=1,25-dihydroxyvitamin D3; NF-κB=Nuclear Factor kappa B; TNF-α=tumor necrosis factor alpha; BDNF= brain-derived neurotrophic factor; PPARγ=peroxisome proliferator-activated receptor-gamma; DMSO=dimethyl sulfoxide; ds DNA=double-stranded DNA; BBB= blood-brain barrier; HE= Hematoxylin and eosin; PBS= phosphate-buffered saline; MS= Multiple Sclerosis

Declarations

Ethical Approval and Consent to participate

All experiments were performed according to the institutional ethical guidelines on animal care and approved by the Institute Animal Care and Use Committee at the Weifang Medical University, Shandong, China.

Consent for publication

The consent of all coauthors was collected before submission.

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

This article is mainly written by XWL and JL. YZ wrote part of the manuscript and proofread the manuscript. RH helped us collect literature information and draw pictures. XLL and YW reviewed the manuscript and proposed final revisions. All authors contributed to the article and approved the submitted version.

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Figures

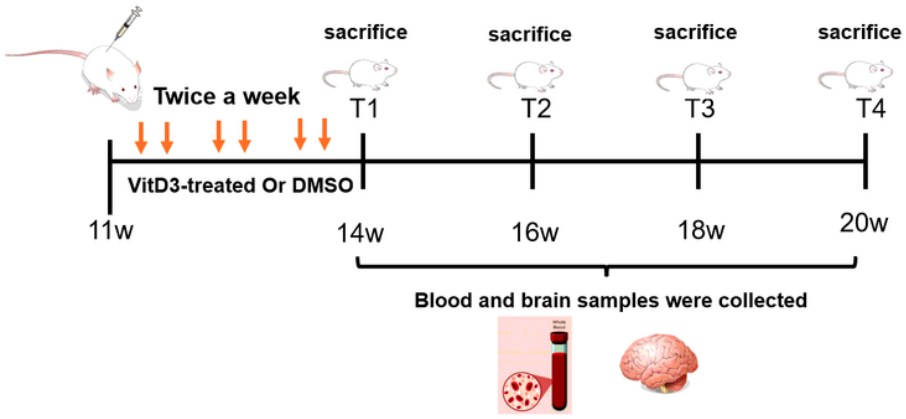

Figure 1

The experiment schedule.

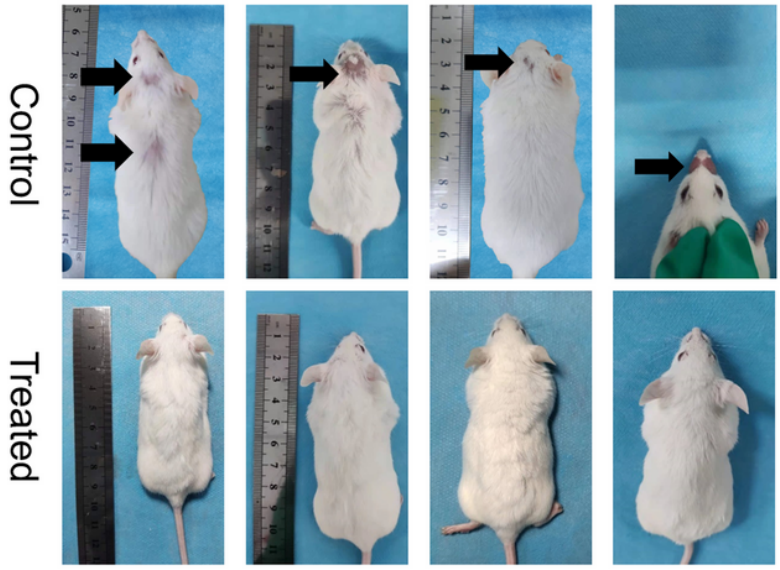

Figure 2

1,25-dihydroxyvitamin D3 inhibits the appearance of skin lesions.

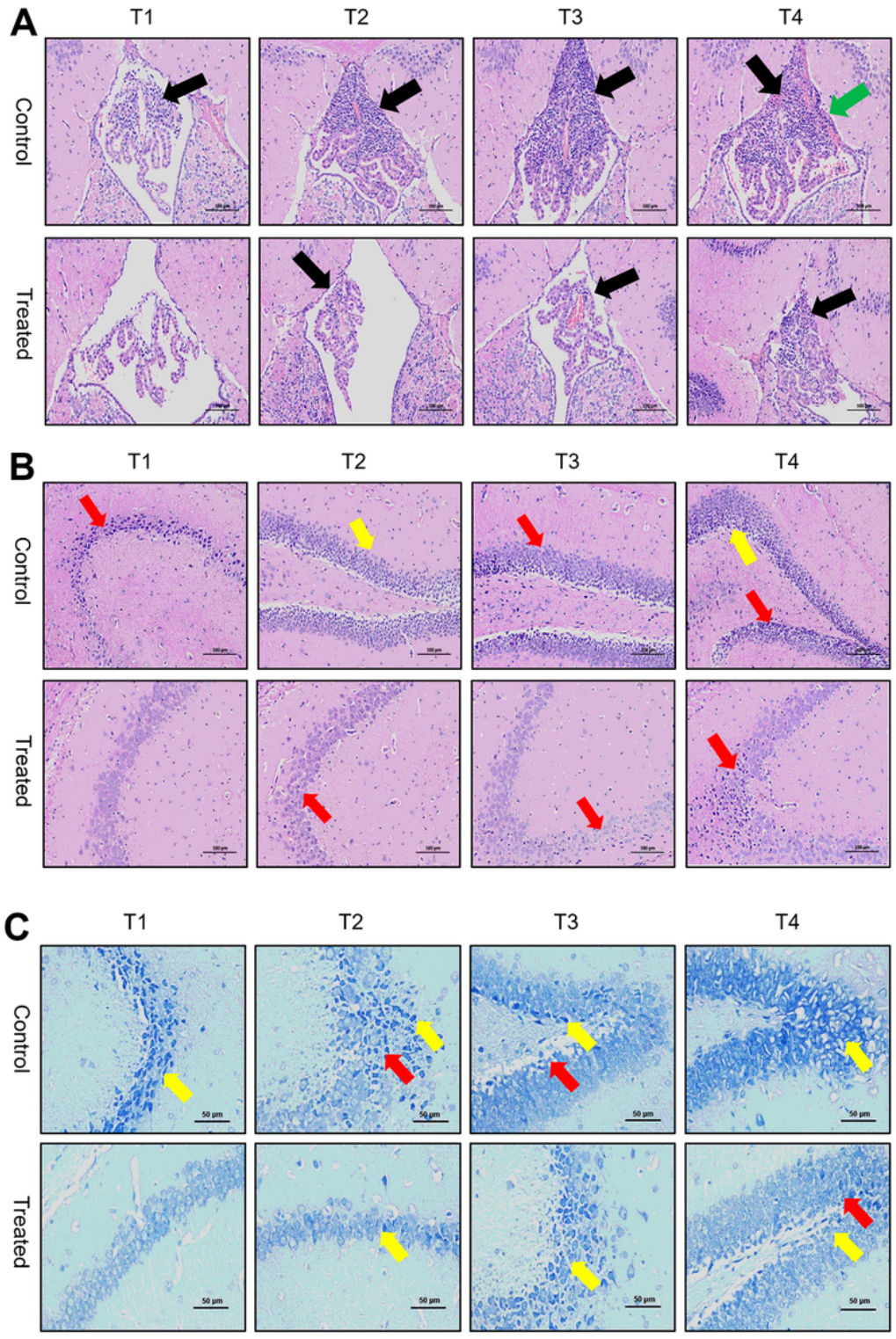

Figure 3

1,25-dihydroxyvitamin D3 ameliorated cerebral injury of NPSLE A. Choroid plexus was observed by HE staining (200 ×); Scale bar=100μm. B. Hippocampus was observed by HE staining (200 ×); Scale bar=100μm. C. Hippocampus was observed by Nissl's staining (400×). Scale bar =50μm.

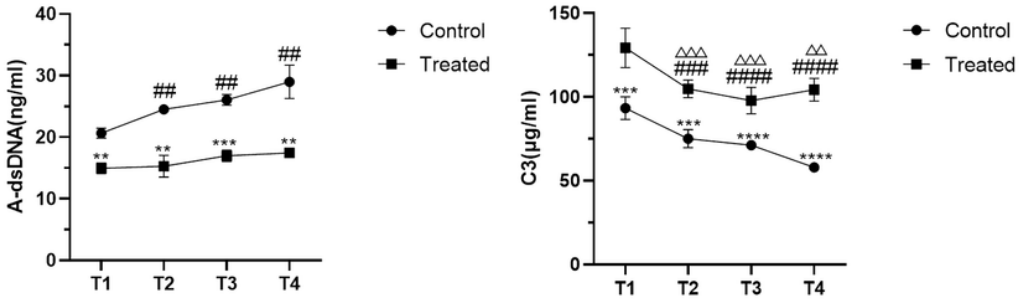

Figure 4

The expression of serum A-dsDNA antibody and C3 complement in MRL/lpr mice at T1 to T4. Values are expressed as means ± SD. * VS. the control group at the same time. # VS. the control group at T1. △VS. the treated group at T1. *#△ p < 0.05, **##△△p < 0.01, ***###△△△p < 0.001，****####△△△△p < 0.0001.

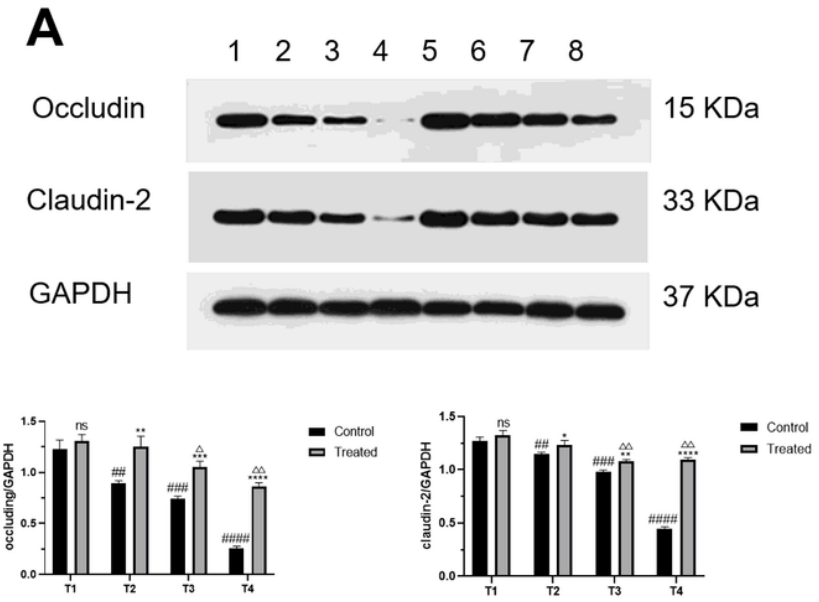

Figure 5

1,25-dihydroxyvitamin D3 protected the BCSFB. The protein expression levels of occludin and claudin-2 by western-blot. Bands 1–8 represent the control groups at T1, T2, T3, T4 and the VitD3-treated groups at T1, T2, T3, T4 respectively. Values are expressed as means ± SD. * VS.
the control group at the same time. # VS. the control group at T1. △VS. the treated group at T1. *#△ p < 0.05, **##△△p < 0.01, ***###△△△p < 0.001, ****####△△△△p < 0.0001.

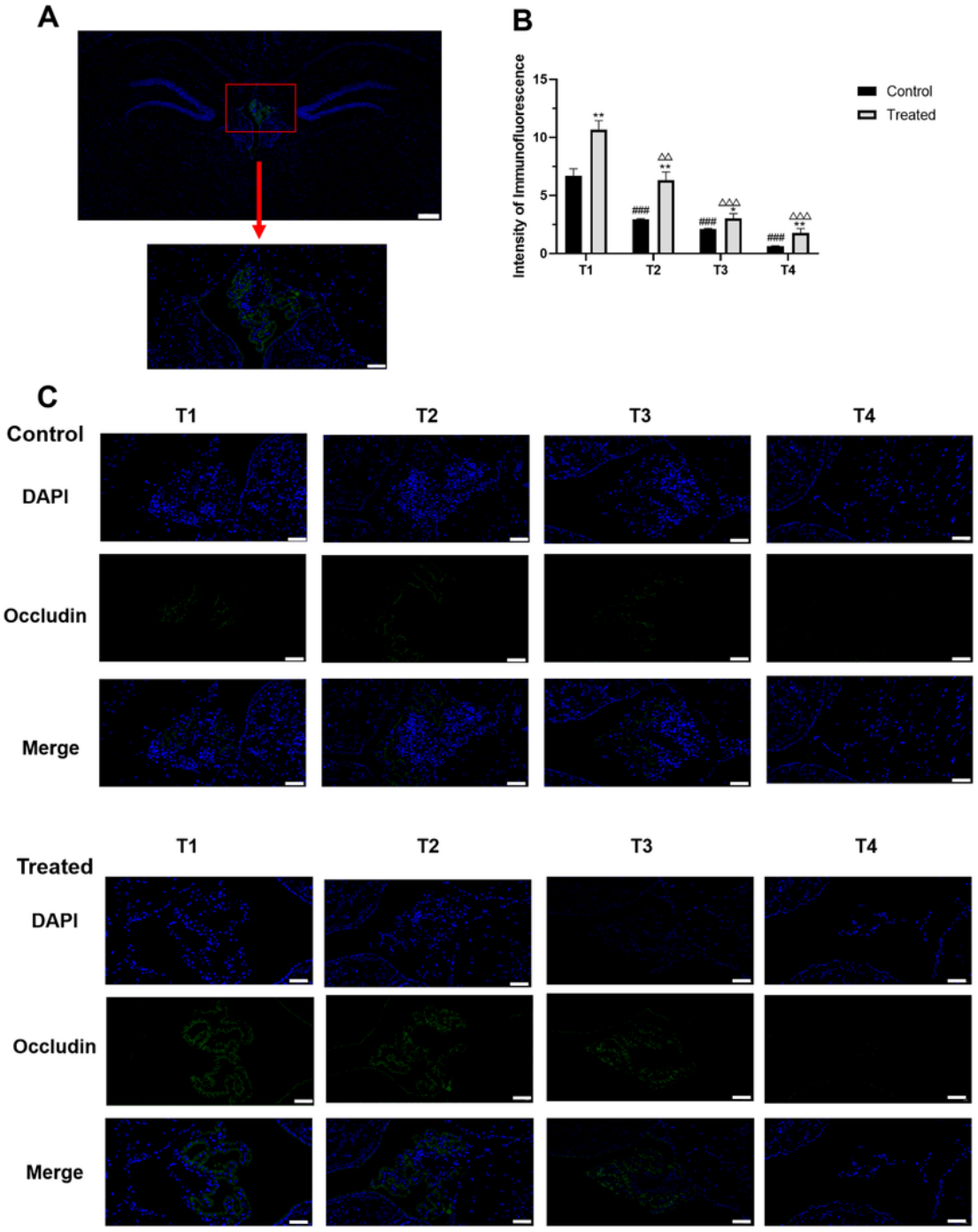

Figure 6

1,25-dihydroxyvitamin D3 protected the BCSFB. A, C. The protein expression levels of occludin by immunofluorescence. B. The intensity of immunofluorescence. Values are expressed as means ± SD. * VS. the control group at the same time. # VS. the control group at T1. △VS. the treated group at T1. *#△ $p < 0.05$, **##△△ $p < 0.01$, ***###△△△ $p < 0.001$, Scale bar=50mm.

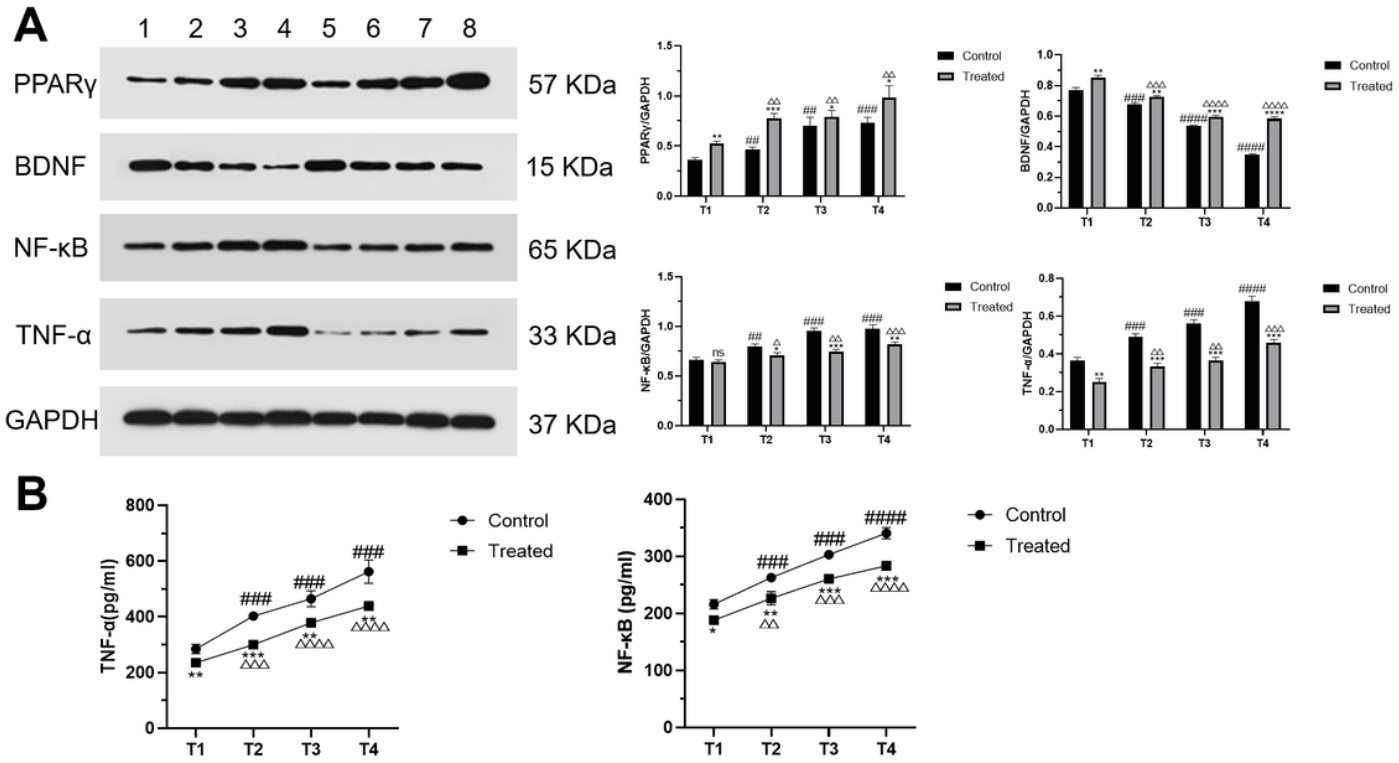

Figure 7

1,25-dihydroxyvitamin D3 suppressed the inflammatory response by activating PPARγ A. The protein expression levels of PPARγ, TNF-α, NF-κB, and BDNF in MRL/lpr mice. B. The expression of serum TNF-α and NF-κB in MRL/lpr. Bands 1–8 represent the control groups at T1, T2, T3, T4 and the VitD3-treated groups at T1, T2, T3, T4 respectively. Values are expressed as means ± SD. * VS.
the control group at the same time. # VS. the control group at T1. △VS. the treated group at T1. *#△ $p < 0.05$, **##△△$p < 0.01$, ***###△△△$p < 0.001$, ****####△△△△$p < 0.0001$.

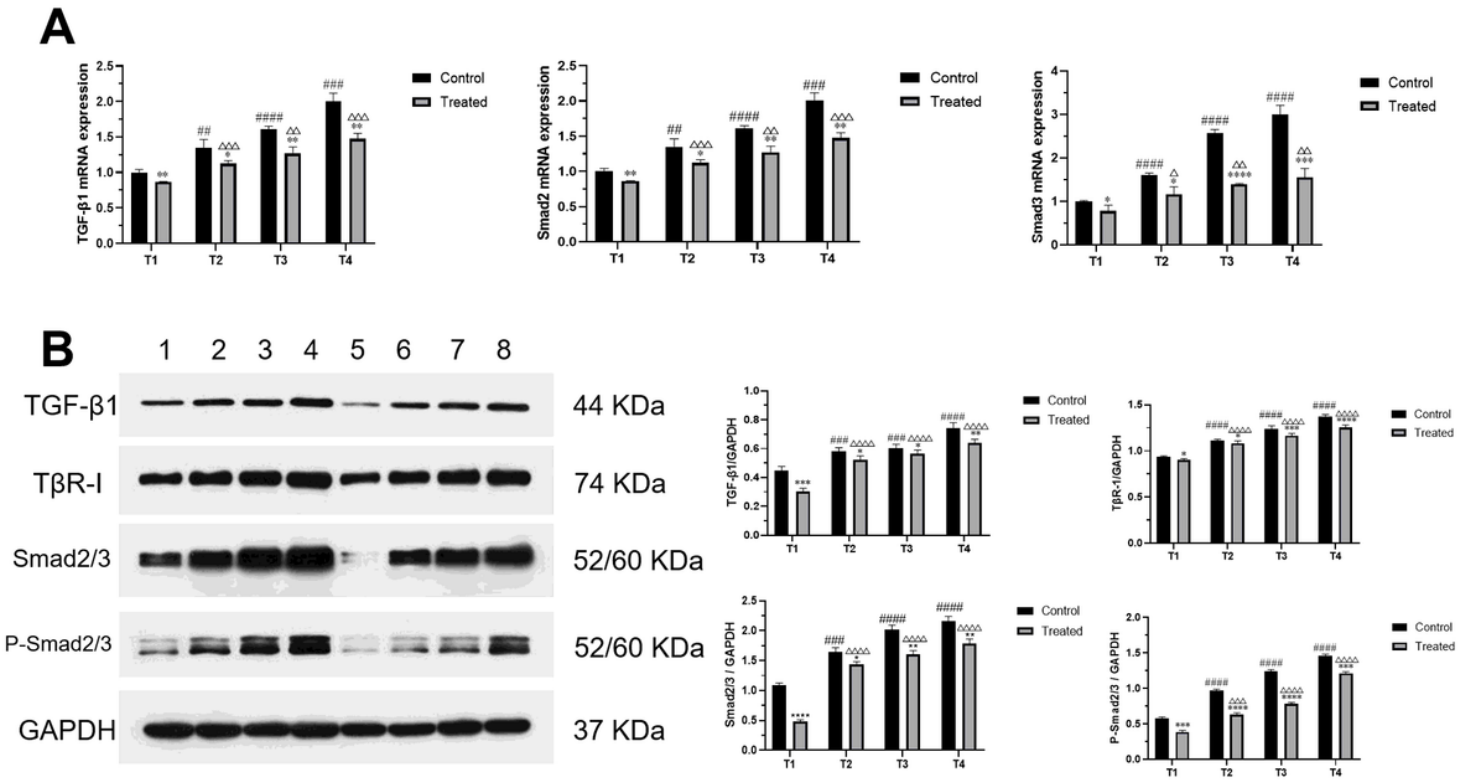

Figure 8

1,25-dihydroxyvitamin D3 inhibited the TGF-β/Smads signaling pathway. A. The mRNA expression levels of TGF-β1、Smad2 and Smad3 in MRL/lpr mice. B. The protein expression levels of TGF-β1、TβR-I, Smad2/3, and P-Smad2/3 in MRL/lpr mice. Bands 1–8 represent the control groups at T1, T2, T3, T4, and the VitD3-treated groups at T1, T2, T3, T4 respectively. * VS. the control group at the same time. # VS. the control group at T1. △VS. the treated group at T1. *#△ $p < 0.05$, **##△△ $p < 0.01$, ***###△△△ $p < 0.001$, ****####△△△△ $p < 0.0001$.