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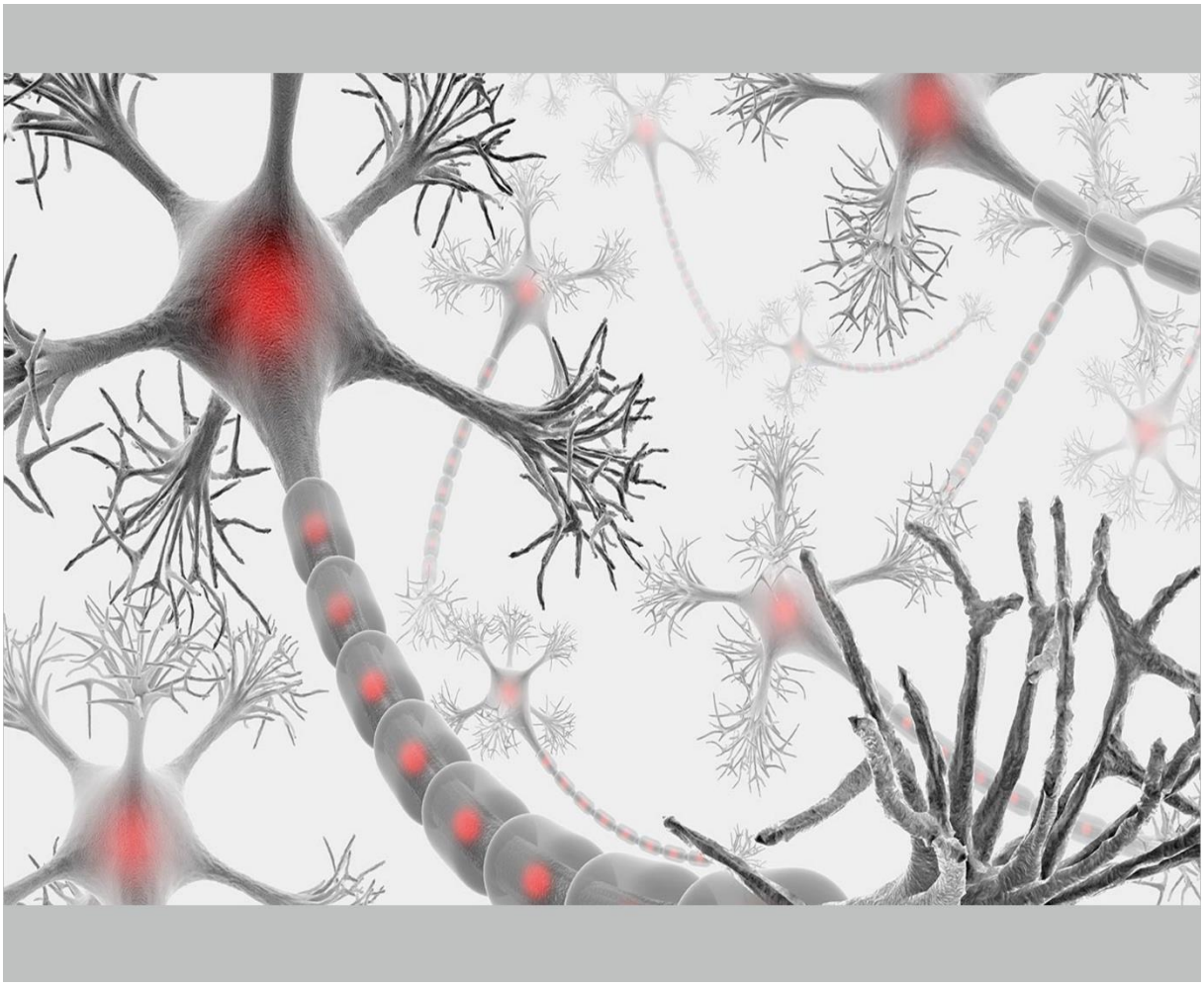
The Potential of Vitamin D in the Treatment of Multiple Sclerosis



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Abstract

Multiple Sclerosis (MS) in an inflammatory and demyelinating disorder of the central nervous system (CNS). The myelinated axons in the CNS are attacked by MS, causing demyelination in various degrees. Victims of MS often suffer from weakness, lack of coordination and impaired speech. Experimental Autoimmune Encephalomyelitis (EAE) in mice is often used as a model for MS and is widely used to study new strategies to control or cure MS. Vitamin D deficiency is highly prevalent in MS patients and recent studies showed that vitamin D deficiency is a risk factor for MS. The intake of vitamin D is mainly through skin exposure to sunlight, but the diet also provides a small percentage of the vitamin. The rate-limiting step in the synthesis of vitamin D is CYP27BI, an enzyme expressed in immune cells. The gene encoding for this enzyme may contribute to MS risk by decreasing the levels of active vitamin D.

Previous studies showed that Myelin Oligodendrocyte Glycoprotein (MOG) in relapsing-remitting MS patients reduced the signs of disease activity furthermore MOG combined with vitamin D therapy downregulated the pro-inflammatory cytokine production. These studies show the potential of vitamin D in the treatment of MS.

In this review, the effects of vitamin D on specific aspects of MS were reviewed, including myelination, tissue lesions, and the immune system. The effect of vitamin D on neural stem cells (NSC) was also overviewed, as presence of NSC in tissue lesions may indicate a repair process.

Results showed that vitamin D can induce remyelination through triggering of the NSC to repair the damaged axons and that vitamin D can suppress apoptosis. However, vitamin D did not downregulate the cytokine production and leads to an increase in body weight and hypercalcemia. A synthetic analog of vitamin D, paricalcitol (PARI), did not lead to an increase in body weight and hypercalcemia.

The data showed that vitamin D is very important in not only reducing the clinical symptoms, but also in triggering NSC differentiation and proliferation. The importance of vitamin D in the disease development implies that individuals who are at risk for MS should be screened for vitamin D deficiency.

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Introduction

Multiple Sclerosis (MS) is a chronic neurological autoimmune disease of the central nervous system (CNS). The myelinated axons in the CNS are attacked by MS, causing demyelination in various degrees. Victims of MS often suffer from weakness, lack of coordination and impaired speech [2]. The development of MS varies and is nearly unpredictable, however it is known that Caucasian women of Northern European descent have the highest risk of developing MS [1].

MS patients can be grouped into four categories: relapsing-remitting MS, secondary progressive MS, primary progressive MS and progressive-relapsing MS[1]. Relapsing-remitting MS is the most common form. It affects 85% of MS patients and is marked by flare-ups of symptoms followed by periods of remission. In secondary progressive MS the symptoms worsen with or without periods of remission. Primary progressive MS affects about 10% of the patients. The symptoms worsen continuously without relapses or remission, but occasional plateaus can occur. Progressive-relapsing MS is the rarer form and it affects less than 5% of the MS patients. The symptoms worsen from the start without relapsing, remission or plateau phases. The cause of MS in yet unknown, but it appears to be a result of genetic and environmental factors. What is known about the development of MS is that demyelination is often caused by self-reactive T-helper-1 (Th1) and T-helper-17 (Th17) cells[7]. The sensory and motor disturbances are a result of demyelination[2].

Myelin sheaths surround the axon of some nerve cells and form an insulating layer[2]. The small sections of the axon that are not insulated by myelin sheaths are called nodes of Ranvier. The main function of myelin is to increase the speed of the electrical impulses. The current “jumps” from node to node over the myelin sheath. Myelin also prevents the current from leaving the axon. It is produced by a certain type of cells, oligodendrocytes. Myelinated nerve cells do not regenerate and demyelination eventually leads to cell death. Demyelination forms lesions in the brain and CNS.

MS can be diagnosed in multiple ways. Lesions in the brain can be viewed through magnetic resonance imaging (MRI) and the velocity of axonal conduction can be measured by electrodes on the scalp[2]. MS patients have a slower conduction due to the loss of myelin, and the latter methods has been used by neurologist for many years.

There is no cure for MS, but there are a number of treatments that can delay the progression (disease-modifying agents) or treat the symptoms, thereby improving the quality of life. Immunomodulatory drugs such as: Interferon beta-1a, interferon beta-1b, glatiramer acetate, mitoxantrone, natalizumab and fingolimod are approved by the FDA[1][3]. The side effects of the beta interferons include liver function abnormalities, leukopenia, thyroid disease and depression. Glatiramer, however, does not cause liver function abnormalities, leukopenia, thyroid disease and depression, but patients report having an itch or a rash, flu-like symptoms, chest pain, hot flashes, and headaches. Although the drugs mentioned can be used to treat relapsing-remitting or secondary progressive MS, they cannot be used to treat primary progressive MS. Most importantly, the drugs are only partially effective. To improve the quality of life symptomatic drugs can be given. Symptomatic treatments include dalfampridine, which has been found to improve the walking speed in patients with various types of MS[4].

It is well known that MS is more prevalent in the northern hemisphere, where the sunlight has a lower intensity that in the southern hemisphere. Recent studies show that an increased skin exposure to sunlight, and therefore a decreased prevalence of vitamin D deficiency, is associated with a decreased risk of developing MS[5]. Low levels of vitamin D also appear to be associated with high levels of disability, which is measured by the Expanded Disability Status Scale (EDSS). The EDSS scale is commonly used as an index of clinical disability in MS. Scores range from 0 (normal) to 10

(death). A study showed that patients with vitamin D levels higher than 50 nmol/L were almost three times more likely to have an EDSS score below 4[8].

Vitamin D is a fat-soluble vitamin that occurs in two main forms: ergocalciferol (vitamin D2) which is of plant origin and cholecalciferol (vitamin D3) which is of animal origin[5]. Vitamin D3 is more bioactive than vitamin D2 and can be obtained from food such as fatty fish. However, the diet provides only a small percentage of the vitamin D intake and the main source is skin exposure to sunlight. In the skin 7-dehydrocholesterol is photolyzed by ultraviolet radiation (UVR) from the sun and converted to pre-vitamin D3. Pre-vitamin D3 is isomerized to vitamin D3. The vitamin D binding protein transports vitamin D3 through the blood to the liver, where vitamin D is hydroxylated by one or more cytochrome P450 vitamin D 25-hydroxylases. This results in the formation of 25-hydroxyvitamin D3 (25(OH)D3), which is the longest living vitamin D metabolite. The status of vitamin D is reflected by the serum levels of 25(OH)D3. The 25(OH)D3 metabolite is further hydroxylated by renal CYP27B1 to 1,25-dihydroxyvitamin D (1,25(OH)2D3) also known as calcitriol, which is the most bioactive vitamin D metabolite. Calcitriol mediates vitamin D signaling by binding to the vitamin D receptor (VDR), which forms a nuclear heterodimer with the retinoid X receptor. The complex can bind to genomic vitamin D response elements, which modulates the expression of genes. Like other hormones vitamin D can also act in fast actions at a cellular level. Vitamin D modulates calcium hemostasis and immunomodulatory functions through these pathways.

Expression of VDR has been reported in most immune cells and CNS tissues. The rate-limiting enzyme for the vitamin D synthesis, CYP27B1, is expressed in immune cells. Therefore, these cells are able to synthesize and secrete active vitamin D, which indicates that vitamin D plays an important role in the immune system. The *in vitro* addition of 1,25(OH)2D3 to antigen-presenting cells inhibits the surface expression of MHC-II and costimulatory molecules leading to reduced T-cell stimulatory capacity. 1,25(OH)2D3 inhibits the production of type 1 Th-cells cytokines (key mediators in autoimmune diseases) and stimulates the production of type 2 Th-cells cytokines, which have immunoregulatory functions. Furthermore, it affects T- and B-cell proliferation and blocks B-cell differentiation and immunoglobin secretion. It also affects T-cell maturation, inducing a shift away from inflammatory Th17 cells phenotype and facilitates the production of regulatory T-cells. All of the immunomodulatory effects can lead to protection of target tissue in autoimmune diseases and transplantation. Experimental studies have reported that the immunomodulatory effects of vitamin D only occur at hyper-physiologic concentrations, which causes hypercalcemia in humans [5].

The largest genome study was completed by the Wellcome Trust Case Control Consortium and the International MS Genetics Consortium. Two genes were identified involved in vitamin D metabolism that could increase the susceptibility to MS[9]. The first is CYP27B1, which encodes for the rate-limiting enzyme in vitamin D synthesis. The second is CYP24A1, which encodes for an enzyme that degrades 1,25(OH)D3. The genes may contribute to MS risk by decreasing the levels of active vitamin D. Vitamin D regulates the RNA expression of the major MS susceptibility gene HLA-DRB1*15:01.

In order to study the effects of vitamin D on the progression of MS, Experimental Autoimmune Encephalomyelitis (EAE) in mice is often used as a model. EAE is an artificially induced CNS disease that resembles the clinical, histopathological and immunological characteristics of MS[7].

In this review, the possible role of vitamin D in the recovery form MS will be discussed. In order to determine the role of vitamin D in MS the effect of vitamin D on tissue lesions, neural stem cells (NSC), the immune system and myelination will be reviewed. The possible advantages of a vitamin D analog will also be discussed.

Effects of vitamin D on tissue lesions and the presence of NSC in damaged tissues

Expression of VDR has been reported in most immune cells and CNS tissues. Recently, VDR was shown to be present in macrophages in chronic active MS lesions[14]. The increased VDR expression in MS lesions suggests an increased sensitivity to vitamin D and a possible role for vitamin D in the suppression of active MS. The effect of vitamin D on brain tissue was analyzed using immunohistochemistry (figure 1-3)[12]. The axons of the control sample show even distribution of myelin. The brain-tissue of non-treated EAE mice shows a loss of axons and signs of demyelination. The brain tissues from the pre-treated EAE mice show less axon damage and less demyelination compared to non-treated EAE mice, and post-treated EAE mice brain tissue shows signs of remyelination.

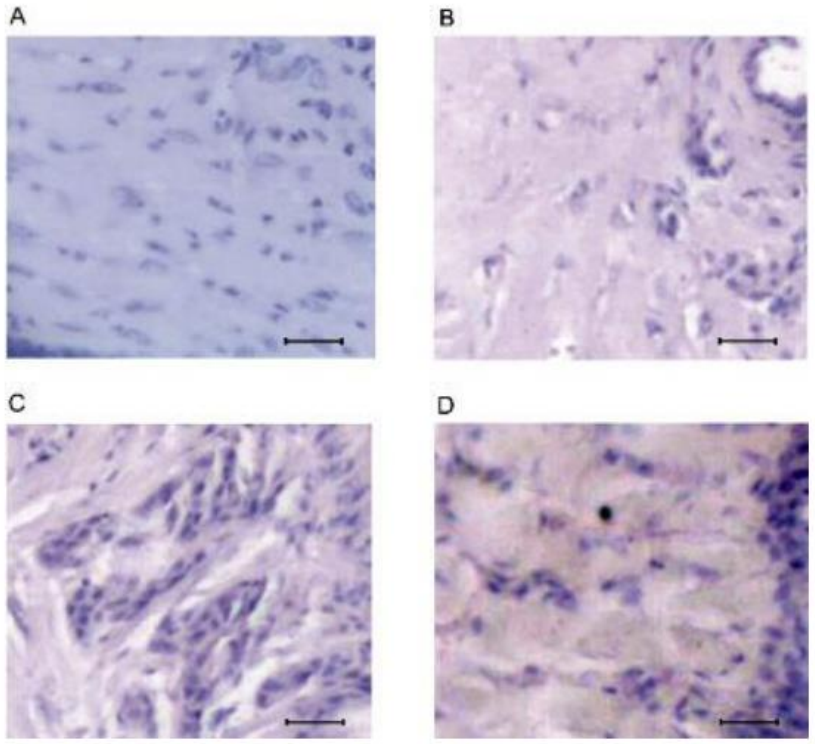

Figure 1: Histological variation between normal, MS and vitamin D gained brain tissue. (A) Histological section of normal brain tissue. (B) Brain tissue from non-treated EAE mice. (C) Brain tissue from pre-treated mice. (D) Brain tissue from pro-treated mice. Scale bar: 50 μm.

NSC play an important role in remyelination and proliferation of the nerve cells. NSC constitutively express VDR. Vitamin D enhances the proliferation of NSC, and enhance their differentiation into neurons and oligodendrocytes[16]. An increase in the NSC count in damaged tissues may lead to remyelination through oligodendrocytes and the formation of new neurons.

Nestin is a protein expressed in NSC and can therefore be used as a marker for the amount of NSC. The results of a brain tissue staining (figure 2) shows that non-treated EAE mice brain tissue have a decreased nestin expression which implies a reduced NSC count. On the other hand, pre-treated EAE mice brain tissue shows increased nestin expression. The increased amount of NSC can imply a repair process after the development of EAE. The pro-treated mice also show signs of repair and proliferation.

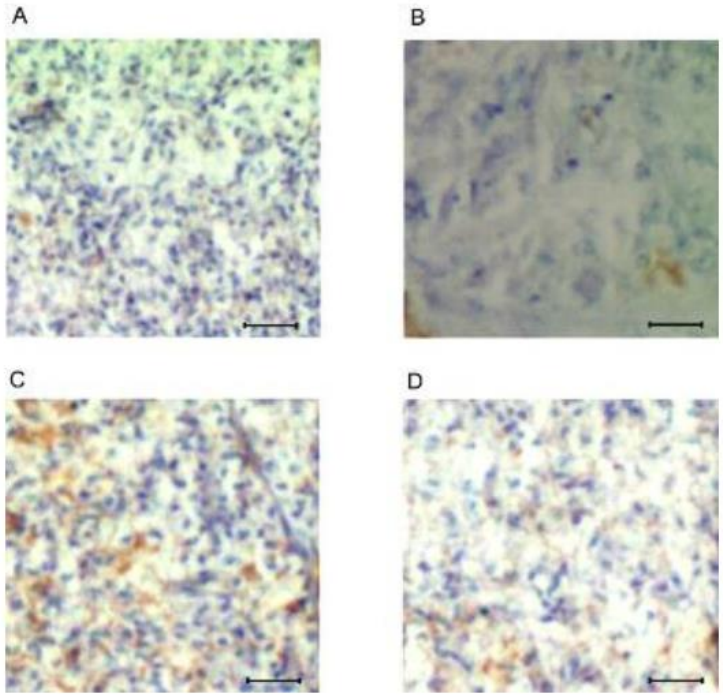

Figure 2: Immunohistological variation associated with NSCs in response to vitamin D. (A) Immunohistological section of normal brain tissue. (B) Brain tissue from non-treated EAE mice. (C) Brain tissue from pre-treated EAE mice. (D) Brain tissue from post-treated EAE mice.

The pathogenesis of MS is far from being well understood, but increasing evidence suggests that inflammatory and apoptotic responses play a role[15]. Caspase-3 can be used as a marker for apoptosis. As mentioned earlier, macrophages express VDR. Macrophages play an important role in inflammatory responses and apoptosis. Vitamin D may have an effect on macrophages and therefore on apoptosis. The immunohistochemistry analysis showed that brain tissue from non-treated EAE mice has an elevated caspase-3 expression. The control sample shows a low caspase-3 expression, which likely indicates a normal regulatory process. Pre-treated brain tissue shows less caspase-3 expression and the post-treated brain tissue shows marginal expression.

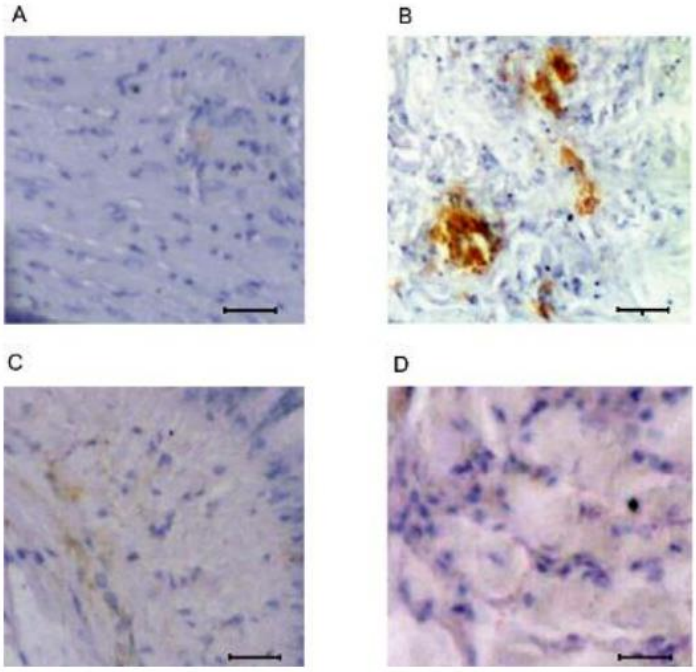

Figure 3: Immonohistological variation associated with an apoptotic protein is response to vitamin D. (A) Immunohistological section of normal brain tissue. (B) Brain tissue from non-treated EAE mice. (C) Brain tissue from pre-treated EAE mice. (D) Brain tissue from post-treated EAE mice.

Western blot analysis revealed that the caspase-3 expression is the highest in non-treated EAE samples, as shown in figure 4B. Pre- and post-treated EAE mice samples show an elevated caspase-3 expression.

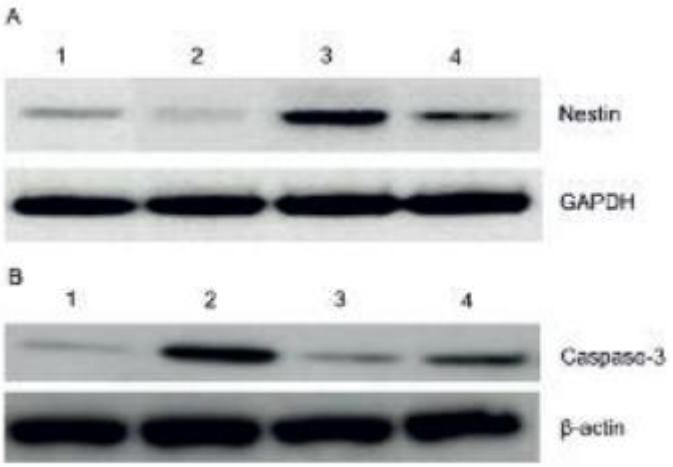

Figure 4: Validation of NSC and apoptotic protein expression by western blotting. (A) Lane 1-4 represents the nestin protein expression in normal, EAE, pre-treated and post-treated brain tissue respectively. (B) Lane 1-4 represents the caspase-3 protein expression in normal, EAE, pre-treated and post-treated brain tissue respectively.

Vitamin D as an immunomodulator

As previously mentioned, inflammatory responses play an important role in the pathogenesis of MS. Cytokines are one of the main components which can induce an inflammatory response. In a recent trial, MS patients were given a patch containing a mixture of 3 myelin peptides, including MOG, and results showed significant effect in reducing the clinical outcomes and MRI lesions [13]. Other studies showed that cytokine production in the spleen of EAE mice submitted to MOG and vitamin D via the intraperitoneal route was down regulated[10].

Paricalcitol (PARI) is a vitamin D analog, which has fewer side effects. PARI may also be able to stimulate VDR similar to vitamin D. Epicutaneous therapy with MOG, PARI or MOG+PARI increased the pro-inflammatory cytokine production by lymph node cell cultures (figure 5). Compared to the control group, all therapies (MOG, PARI and MOG+PARI) significantly increased the cytokine production.

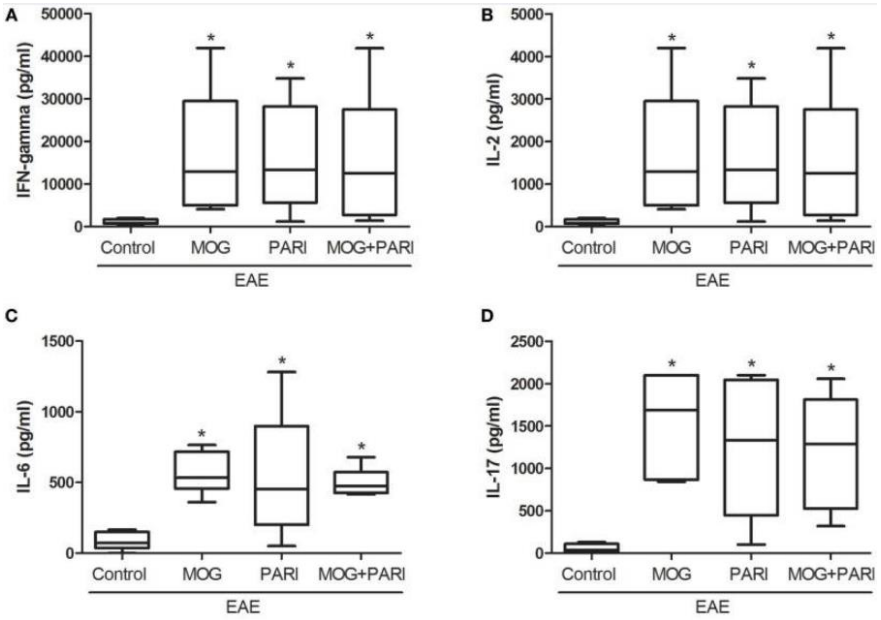

*Figure 5: Effect of MOG+PARI association on cytokine production. This figure shows the concentration of (A) IFN- γ, (B) IL-2, (C) IL-6 and (D) IL-17 in pg/mL. Significant differences are marked by *.*

Next to the inflammatory cytokines, the regulatory immune profile was also affected by the therapy. This profile was characterized by high levels of TGF-β, Foxp3+ Treg cells and lower levels of MHCII fluorescence intensity (figure 6). The IL-10 levels were not significantly increased or decreased in comparison to the EAE control group.

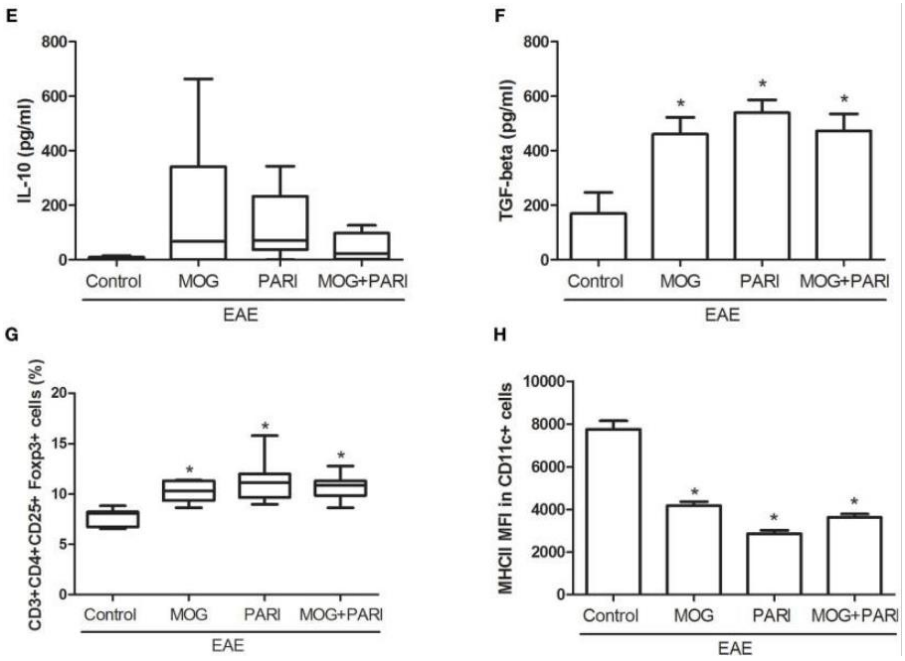

*Figure 6: Effect of MOG+PARI association on cytokine production and immune cells. This figure shows the concentration of (E) IL-10, (F) TGF-beta in pg/mL. This figure also shows the (G) CD3+ CD4+ CD25+ Foxp3+ and (H) MHCII MFI in CD11+ cell count. Significant differences are marked by *.*

Effects of vitamin D on myelination

EAE is characterized by infiltration of immune cells in the CNS and predominant demyelination of axons in the acute phase of the disease. The therapeutic effect of MOG+PARI could be related to a milder CNS pathology. Lumbar spinal cord sections were stained and analyzed. The results are shown in figure 7[7]. The EAE group showed a pronounced inflammatory process in the meninges and in the spinal cord parenchyma, as shown in figure 7C. MOG and MOG+PARI treatments significantly reduced the extension of the inflammatory infiltrates as shown in figure 7D and F. The degree of inflammation in the lumbar spinal cord of PARI treated mice was similar to the non-treated mice (figure 7E). As shown in figure 7K, MOG and MOG+PARI conserved myelin sheaths whereas PARI treatment showed areas with demyelination (figure 7J).

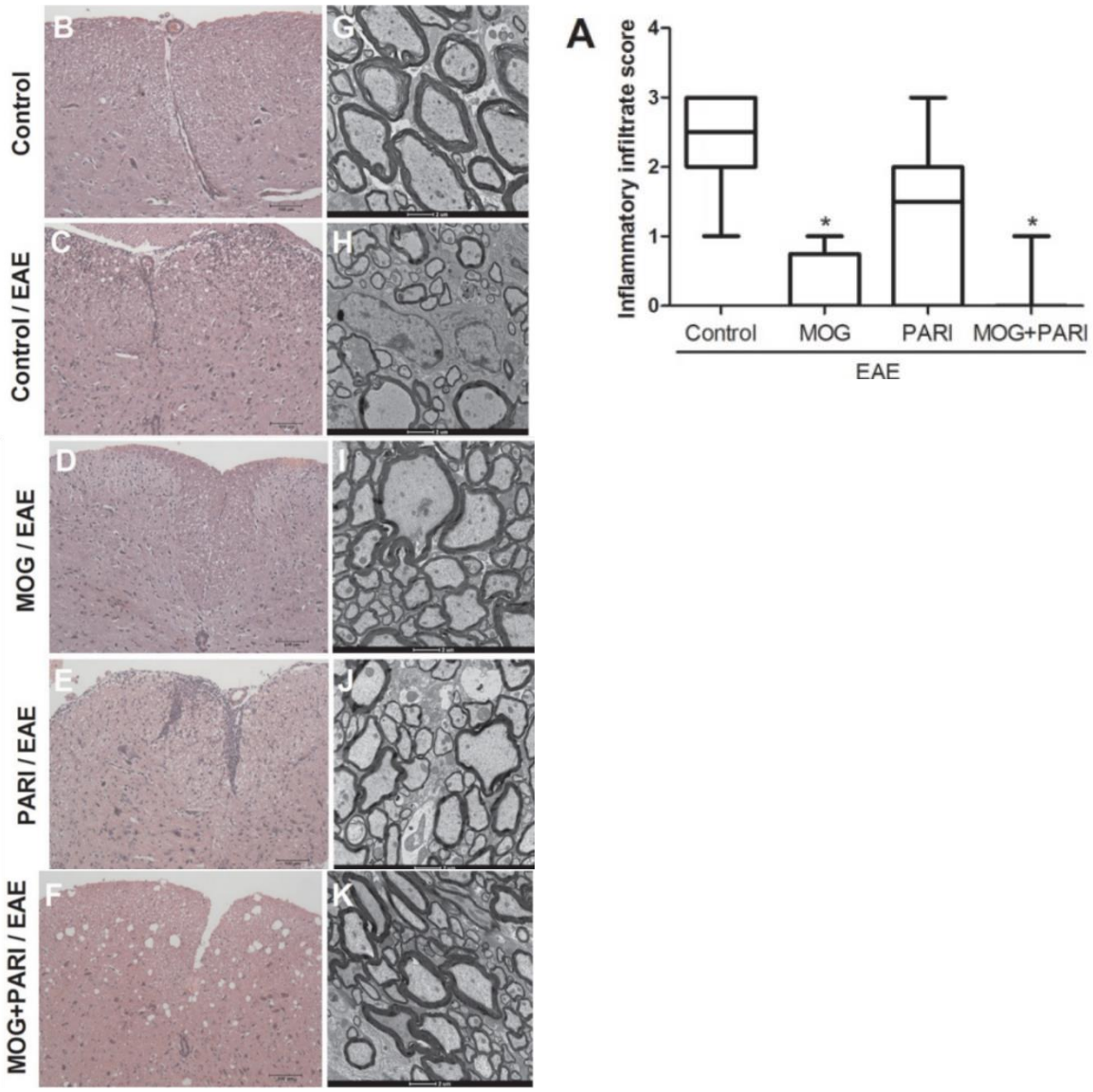

Figure 7: Control of inflammation and demyelination in the central nervous system by MOG+PARI association. (A) a semiquantitative analysis was used to assess the inflammatory infiltration in the acute EAE phase. (B-F) sections were stained with hematoxylin and eosin. (G-K) areas of lumbar spinal cord were used to assess myelin sheath swelling using transmission electron microscopy.

Vitamin D is able to reach the CNS and interact with microglial cells[11], and PARI was expected to have similar characteristics. As shown in figure 8B[7], the microglial cells eluted from the CNS of mice treated with PARI or MOG+PARI showed a significant reduction in the MFI of MHCII. CD40 (figure 8A) and PD-L1 (figure 8C) MFI expression were not affected. Addition of PARI or MOG+PARI to BV-2 cells gives a similar result (figure 8E). Figure 8D and F shows a significant reduction in CD40 and PD-L1 respectively. MOG+PARI association decreased IL-6 (figure 8H) and NO (figure 8I) and increased IFN-γ (figure 8G) and IL-10 (figure 8I).

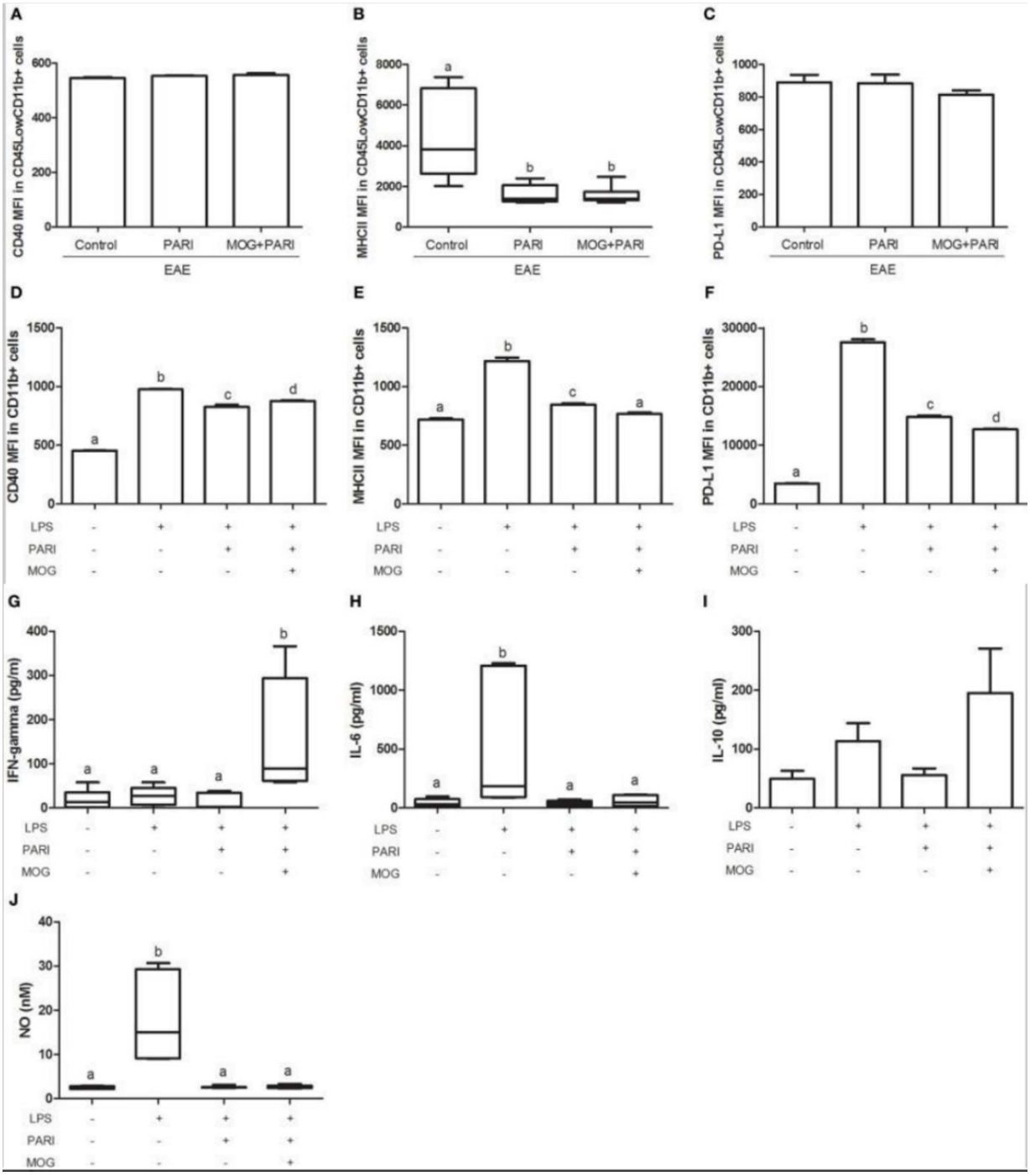

Figure 8: MOG+PARI association modulates microglial cells. (A-C) microglial cells from the CNS at the acute EAE phase. (D-J) In vitro LPS activated BV-2 cell line after pretreatment with PARI and MOG.

Advantages of a vitamin D analog

The effect of PARI, a synthetic vitamin D analog, on EAE development was compared to that of vitamin D3 (figure 9 and 10)[7]. Results show that the administration of MOG and PARI or vitamin D3 by the same route, either intraperitoneal or epicutaneous, was more efficient to control the development of EAE than administration of the components by different routes. When epicutaneous MOG was administered with ip PARi or VitD, the disease incidence is still high at around 50%. However, administration of MOG+PARI via the epicutaneous route and administration of MOG+VitD via the intraperitoneal route resulted in a disease incidence of 0%. Figure 9 also shows that PARI does not trigger body weight loss.

Groups	Disease incidence (%)	Maximum clinical score	Weight variation (%)
Control / EAE (n=5)	100	2 (1.5 - 3.5)	- 4.8 +- 7.7
MOG epi + PARI ip / EAE (n=6)	50 (p=0.182)	0.5 (0 - 2.5)	+ 0.5 +- 11.5
MOG epi + VitD ip / EAE (n=6)	50 (p=0.182)	0.5 (0 - 1.25)	- 9.3 +- 7.5
MOG ip + PARI ip / EAE (n=6)	33.3 (p=0.061)	0 (0 - 2) *	+ 1.8 +- 8.2
MOG ip + VitD ip / EAE (n=6)	0 * (p=0.002)	0 (0 - 0) *	- 18.1 +- 2.3 *
MOG epi + PARI epi / EAE (n=6)	0 * (p=0.002)	0 (0 - 0) *	+ 7.5 +- 3.9 *
MOG epi + VitD epi / EAE (n=6)	16.7 * (p=0.015)	0 (0 - 0.25) *	+ 6.4 +- 4.6 *

* p< 0.05 versus control

Figure 9: Effect of MOG+PARI or MOG+VitD on EAE development. This figure shows the disease incidence, maximum clinical score and weigh variation in both the control and the treated mice.

The data in figure 10 shows that the serum calcium levels after the treatment with vitamin D3 are significantly elevated relative to the control group. The serum calcium levels of the PARI treated mice are not significantly elevated. This suggests that treatment with vitamin D3 will result in hypercalcemia, whereas treatment with PARI does not.

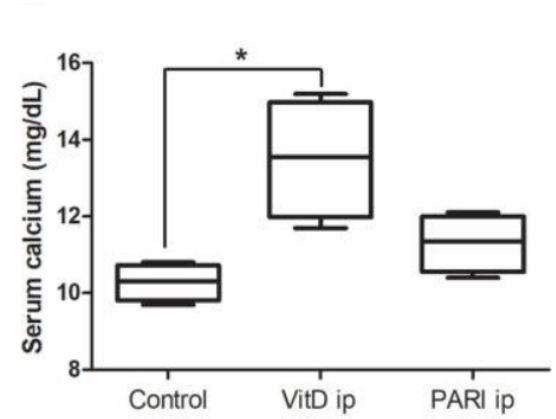

Figure 10: Serum calcium 1 day after the treatment with either VitD or PARI. This figure shows the serum calcium concentration (in mg/mL) for the control group and the mice treated with either VitD or PARI. Both VitD and PARI were administered via the intraperitoneal route.

It is hypothesized that administration of PARI would improve the MOG efficacy when the two substances were co-administered. The results show that co-administration of MOG+PARI, both by the epicutaneous route, results in a much milder disease in comparison with MOG or PARI administration alone (figure 11)[7]. The clinical score of the control group is highest 20 days after EAE induction, with a clinical score of almost 5. The clinical scores of PARI and MOG at 20 days after EAE induction are around 2. Co-administration of MOG and PARI result in the lowest clinical score, which is below 1. The protective effect of co-administration is supported by not only lower clinical scores, but also by no body weight loss (figure 12) and a clear decreased disease incidence (figure 13).

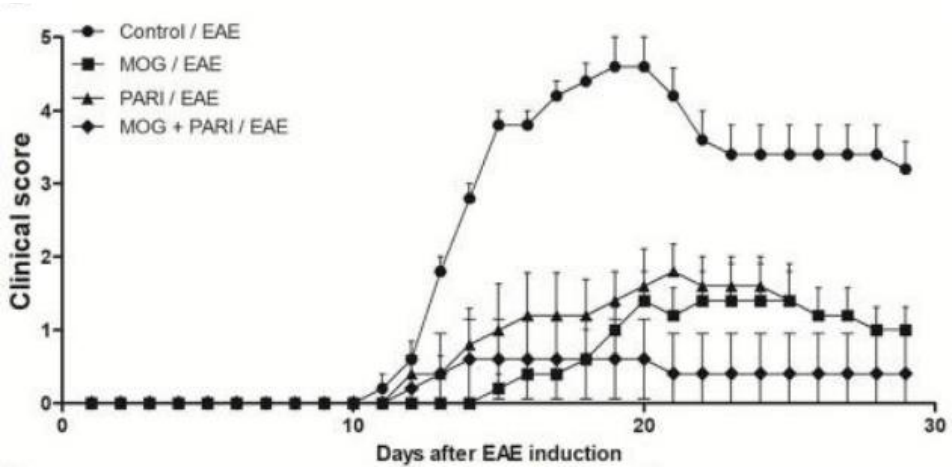

Figure 11: Clinical score of the control and treated EAE mice. This figure shows the clinical score on the y-axis and the days after EAE induction on the x-axis.

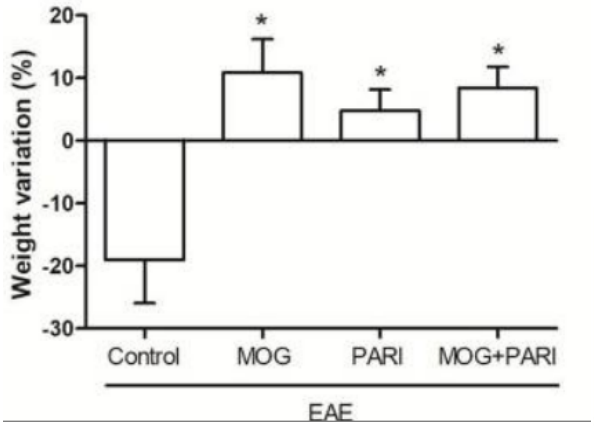

*Figure 13: Weight variation in control and treated mice. This figure shows the weight variation in percentages on the y-axis and the treatment on the x-axis. Significant differences are marked with *.*

Incidence	Sick number	Sick (%)	
Control / EAE (n=21)	21	100%	p<0.001
MOG / EAE (n=17)	8	47%	
PARI / EAE (n=28)	12	43%	
MOG + PARI / EAE (n=33)	7	21%	

Figure 12: Disease incidence. This figure shows the disease incidence in the control and treated groups.

Discussion and conclusion

This review focused on vitamin D as a potential treatment for MS. The effects of vitamin D on tissue lesions, the immune system, myelination, and the presence of NSC in tissue lesions. Furthermore, the possible advantages of a vitamin D analog were analyzed in order to give the proper conclusion.

The pathogenesis of MS seems to be linked to an inflammatory response. The VDR expression on immune cells and NSCs make vitamin D a promising option for treatment. Vitamin D was shown to promote remyelination in post-treated EAE mice and reduce the damage to axons in pre-treated EAE mice. Vitamin D also promotes migration of NSCs to the damaged brain tissues, and reduces the amount of apoptosis. By the reparation of tissue lesions, the clinical symptoms of MS can possibly reduce. The next important step in the treatment of MS would be to downregulate the inflammatory response in order to prevent tissue lesions and infiltration of immune cells in the CNS.

Cytokines, the key to an inflammatory response, can also be mediated by vitamin D. Treatment with vitamin D and MOG showed to be effective in downregulating the cytokine release and upregulating the regulatory immune profile. The infiltration of immune cells in the CNS was reduced after vitamin D/PARI treatment. PARI, a synthetic vitamin D analog, was shown to be even more effective in regulating the cytokine release. PARI did not, contrary to vitamin D, trigger body weight loss. Furthermore, PARI did not increase the serum calcium levels. These findings make PARI a more suitable treatment for MS than vitamin D. PARI is currently used in kidney patients to treat and prevent high levels of parathyroid hormone and is also experimentally given to patients who suffer from cardiovascular diseases, diabetic nephropathy, myelodysplastic syndrome, pancreatic cancer, pentylenetetrazol-induced seizures or post-transplantation nephropathy[17][18].

In summary, the data demonstrates that epicutaneous administration of MOG+PARI was highly effective to control EAE development. This makes it a promising treatment for MS patients, as the results also showed remyelination. The main point of focus is NSC, because NSC is the basis for tissue repair as it has the ability to proliferate and differentiate into neurons. Vitamin D was able to promote the proliferation of NSC and it can improve clinical symptoms. *In vitro* studies prove its role in controlling cell growth, hindering DNA damage and it has a therapeutic role in breast, colon and skin cancer. Pre-treatment with vitamin D showed positive effects in triggering NSC and thereby preventing the development of complex stages of EAE when compared to the positive control.

In conclusion, vitamin D is very important in not only reducing the clinical symptoms, but also in triggering NSC differentiation and proliferation. The data also showed that PARI, a vitamin D analog, is just as effective as vitamin D, and does not lead to hypercalcemia. The main problem is that EAE, and even though it is similar to MS in humans, it is not the same. As previously mentioned, MS patients have a high risk of developing a vitamin D deficiency. The data showed the importance of vitamin D to control the disease development and therefore individuals who are at risk for MS should be screened for vitamin D deficiency.

The effect of high doses of vitamin D/PARI in MS patients is not yet known and should therefore be studied before vitamin D/PARII treatment is applied to MS patients.

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