1, 25-dihydroxyvitamin D₃ (VD3) enhances neural stem cell proliferation & oligodendrocyte differentiation

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Investigation Objectives

- Evaluation of interaction between Vitamin D₃ and neural stem cell proliferation and differentiation in to oligodendrocyte as the myelinating cell

- Assessment of Vitamin D₃ effect on Multiple Sclerosis progression
Multiple Sclerosis

- Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system.

- Immunologic, infectious, genetic factors and environmental risk factors like insufficient exposure to the sunlight and naturally-produced of vitamin D3.
**Vitamin D**

- Regulation of calcium, phosphorus, bone metabolism and neuromuscular function

- Regulation of immune responses attaching to a specific receptor (VDR) in cerebral white matter and inhibiting chronic inflammatory disease

- Regulation of cell proliferation and differentiation
1,25 (OH)_2D_3 and Oligodendrocyte

🌟 Oligodendrocyte in brain and spinal cord expresses vitamin D receptor (VDR)

🌟 Lack of level 1,25-(OH)_2D_3 ➔ Decreasing of differentiation and axonal adhesion of oligodendrocyte ➔ increasing oligodendrocyte apoptosis ➔ immune system disorder like MS

🌟 Oligodendrocyte apoptosis and dystrophy are important pathological features in demyelinating lesions of MS
Analysis of proliferation and differentiation

In vitro

In vivo
VD3 on NSC Proliferation on Days 1, 3, 5, 9, 14

- 1,25(OH)₂D₃ induced higher proliferation with concentration 1 × 10⁻⁴ M on Day 14
- Neurospheres were capable of secondary expansion

Day 9
- Control
- 1,25(OH)₂D₃

Day 14
- Control
- 1,25(OH)₂D₃

Result

- 1,25(OH)₂D₃ 1 × 10⁻¹⁰ – 1 × 10⁻⁴ M
- Days 1 - 14

*P<0.05
Result: The best concentration of 1,25(OH)\(_2\)D\(_3\) considered 1 \(\times 10^{-4}\) M
Result

Enhanced differentiation with detected concentration $1 \times 10^{-4}$ M of $1,25(\text{OH})_2\text{D}_3$
VD3 on immunoreactivity in NSC

Neurosphere

![Image of Neurosphere](image1)

![Image of GALC](image2)

![Image of DAPI](image3)

![Image of Merge](image4)

![Graph showing VD3 effect on markers](graph)

**GALC**  
**DAPI**  
**Merge**

Control  
VD3

Markers: GALC, Nestin, Nestin, GFAP

Cells %

- Control
- 1,25(OH)2D3

*P < 0.05*
Immunization and EAE Induction

- C57BL/6 (8-10 weeks)
- Immunization and induction of EAE (Experimental Autoimmune Encephalomyelitis)

- Myelin Oligodendrocyte Glycoprotein (MOG) 200 µg and Complete Freund’s Adjuvant (CFA) subcutaneous injection
- Pertussis toxin (200 ng) intraperitoneal injection (i.p) at the time of immunization and 48 hours later
Treatment with 1,25-(OH)2-D3

- **Treated animals**

  Injection 1,25-(OH)2-D3 (0.10 µg in 0.1% ethanol) in normal saline every other day for 15 days

- **Control animals**

  Injection 0.1% ethanol in normal saline every other day for 15 days
Disease activity assessment

- Animals were examined daily for signs of disease and graded on a scale of 0 to 5 of increasing severity of disease.
VD3 on Inflammation

EAE

Spinal cord

VD3 treated

![H&E images](image)

**Spinal Cord**

![Bar chart](chart)

- Control
- 1,25(OH)₂D₃

*P<0.05

Inflammatory cells

- 0
- 10
- 20
- 30
- 40
VD3 on Demyelination

EAE

Spinal cord

VD3 treated

LFB

Demylinated area cells

Spinal Cord

Control

1,25(OH)_{2}D_{3}

*P<0.05

Demylinated area cells

Control

1,25(OH)_{2}D_{3}
VD3 on Immunoregulation

**Spleen**

- **Control**
- **1,25-(OH)2D3**

**Cytokines**

- IL4
- IL5
- IL10
- IL17
- GMCSF
- IFN

**CNS**

- **Control**
- **1,25-(OH)2D3**

**Cytokines**

- IL4
- IL5
- IL10
- IL17
- GMCSF
- IFN

**Graphs:**

- **Spleen**
- **CNS**

- *P < 0.05

**Legend:**

- Control
- 1,25-(OH)2D3

**CD4 Positive Cells %**

- **Spleen**
- **CNS**

- IL4, IL5, IL10, IL17, GMCSF, IFN
VD3 on Cytokine levels & Immunoassay

Spleen

CNS
VD3 on mRNA expression

Spleen

CNS

* P< 0.05

Vehicle
1,25 (OH)2D3

Relative expression level
VD3 on immunoreactivity

Spinal cord

Nestin

Number of cells %

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>1,25(OH)2D3</th>
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<tbody>
<tr>
<td>CD3</td>
<td></td>
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<tr>
<td>CD11b</td>
<td></td>
<td></td>
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<tr>
<td>Nestin</td>
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<tr>
<td>GALC</td>
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<tr>
<td>NG2</td>
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VD3

Control

Merge
VD3 on immunoreactivity
Spinal cord

Number of cells %

<table>
<thead>
<tr>
<th>Cells</th>
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<th>1,25,(OH)2D3</th>
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<tbody>
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<td>CD4</td>
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<td><img src="image3" alt="Graph" /></td>
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<tr>
<td>NG2</td>
<td><img src="image9" alt="Graph" /></td>
<td><img src="image10" alt="Graph" /></td>
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Oligodendrocyte

GALC  DAPI  Merge

Control

VD3

Bar scale: [Bars]
VDR detection in Oligodendrocyte

Spinal cord

Oligodendrocyte & VDR

Merge
VD3 on immunoreactivity

Spinal cord

CD4

Control

VD3

CD4

DAPI

Merge
VD3 on immunoreactivity

Spinal cord

CD11b

Number of cells %

Control

1,25(OH)2D3

CD11b

DAPI

Merge

Control

VD3
VD3 on immunoreactivity

Spinal cord

Control

VD3

OPC

DAPI

Merge
Summary

- This study provides evidence for strong association of vitamin D₃ with development of MS and EAE progression suggesting as a neuroprotective factor and possessing of potential effects on NSC proliferation and oligodendrocyte differentiation.

- Vitamin D₃ advantages are selective immunosuppression of autoimmune cells, the positive regulation of other immune cells and ease of delivery that might decrease the risk of infection, so can be suggested as a novel and effective treatment promoting endogenous remyelination.

- Moderate sun exposure and vitamin D supplement can be effective complements for MS prevention and management.
Acknowledgment

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Thank you