



Vitamin D Modulates GAP-43 And STMN2 Expression After Axonal Injury

P-1273

Ebru Keskin¹, Duygu Gezen-Ak², Erdinç Dursun²

¹ Istanbul University-Cerrahpaşa, Department of Neuroimmunology, Institute of Neurological Sciences, Istanbul, Turkey

² Istanbul University-Cerrahpaşa, Brain and Neurodegenerative Disorders Research Laboratories, Department of Neuroscience, Institute of Neurological Sciences, Istanbul, Turkey

Vitamin D may enhance axonal regeneration by upregulating GAP-43 and STMN2 expression after axonal injury.

INTRODUCTION

Axonal regeneration in the central nervous system (CNS) is limited by both intrinsic and extrinsic mechanisms [1]. However, stimulation with appropriate neurotrophic factors has been shown to promote axonal regeneration in the CNS to a limited extent [2, 3]. In an animal model of peripheral nerve injury, vitamin D has been reported to increase the number of newly formed axons and axonal diameter at the distal end, as well as enhance neurite myelination at both distal and proximal sites [4]. During developmental and regenerative processes, axonal elongation is associated with high expression levels of the axonal growth-associated proteins GAP-43 and STMN2[5, 6].

The literature contains limited data on the role of vitamin D in axonal regeneration within the central nervous system. In this context, the present study aimed to examine whether vitamin D administration following axonal injury in mature neurons modulates the expression of GAP-43 and STMN2 and exerts a neuroprotective effect after injury.

METHODS

Our study was performed in primary cortical neuron cultures derived from embryonic day 16 rat embryos, a model widely regarded as the gold standard for investigating neuronal maturation and functional activity.

On day 7 of culture (DIV 7), an axonal injury model known as the replating assay was established by detaching and replating mature neurons.

Following treatment with 10^{-8} M 1,25-dihydroxyvitamin D₃ or ethanol after axonal injury, GAP43 (Figure 1) and STMN2 (Figure 2) protein expression levels were evaluated at 24, 48, and 72 hours by Western blot analysis and immunofluorescence labeling.

RESULTS

GAP43 expression levels were significantly increased in the vitamin D group at 24h and 48h compared to the ethanol group ($p < 0.001$, $p < 0.05$, respectively). STMN2 expression was also significantly elevated in the vitamin D group at 24h relative to the ethanol group and at 48h compared to the control group ($p < 0.001$, $p < 0.05$, respectively). In contrast, at 72h, both GAP43 and STMN2 expression levels were significantly reduced in the vitamin D group compared to the ethanol group ($p < 0.0001$, $p < 0.01$, respectively). The immunofluorescence intensities of GAP43 and STMN2 proteins were validated using corrected total cell fluorescence (CTCF) analysis.

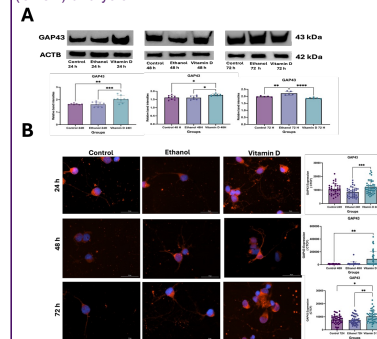


Figure 1. Effects of vitamin D treatment on GAP-43 protein expression following axonal injury. Primary cortical neurons undergoing an axonal injury model were treated with ethanol or 10^{-8} M 1,25-dihydroxyvitamin D₃ and protein expression levels were evaluated at 24, 48, and 72 h post-injury. (A) Western blot analysis and quantification of GAP-43 protein expression. (B) Immunofluorescence staining of GAP-43 and corrected total cell fluorescence (CTCF) analysis. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

CONCLUSIONS

The findings of this research suggest that vitamin D administration following an axonal injury in mature neurons promotes the expression of GAP-43 and STMN2, which may facilitate the process of axonal regeneration.

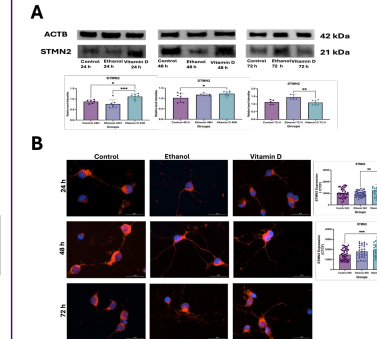


Figure 1. Effects of vitamin D treatment on STMN2 protein expression following axonal injury. Primary cortical neurons undergoing an axonal injury model were treated with ethanol or 10^{-8} M 1,25-dihydroxyvitamin D₃ and protein expression levels were evaluated at 24, 48, and 72 h post-injury. (A) Western blot analysis and quantification of STMN2 protein expression. (B) Immunofluorescence staining of STMN2 and corrected total cell fluorescence (CTCF) analysis. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

REFERENCES

1. Fawcett, J.W., *The Struggle to Make CNS Axons Regenerate: Why Has It Been so Difficult?* Neurochem Res, 2020, 45(1): p. 144-155.
2. Grill, R., et al., *Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury.* J Neurosci, 1997, 17(14): p. 5560-72.
3. Tetzlaff, W., et al., *Response of rubrospinal and corticospinal neurons to injury and neurotrophins.* Prog Brain Res, 1994, 103: p. 271-86.
4. Chabas, J.F., et al., *Cholecalciferol (vitamin D3) improves myelination and recovery after nerve injury.* PLoS One, 2015, 8(5): p. e05034.
5. Mason, M.R., et al., *Transcriptional upregulation of SCG10 and GAP-23 is correlated with regeneration of the axons of peripheral and central neurons in vivo.* Mol Cell Neurosci, 2002, 20(4): p. 595-615.
6. Skene, J.H., et al., *A protein induced during nerve growth (GAP-43) is a major component of growth-cone membranes.* Science, 1986, 233(4765): p. 783-6.

CONTACT

ebru.keskin@iuc.edu.tr

<https://www.linkedin.com/in/ebru-keskin>

