NEURAL REGENERATION RESEARCH
May 2016, Volume 11, Issue 5
www.nrronline.org

RESEARCH ARTICLE

Vitamin B complex and vitamin B_{12} levels after peripheral nerve injury

Idiris Altun[1,*], **Ergül Belge Kurutaş**[2]

1 Department of Neurosurgery, Kahramanmaras Sutcu Imam University Medical Faculty, Kahramanmaras, Turkey
2 Department of Biochemistry, Kahramanmaras Sutcu Imam University Medical Faculty, Kahramanmaras, Turkey

How to cite this article: *Altun I, Kurutaş EB (2016) Vitamin B complex and vitamin B_{12} levels after peripheral nerve injury. Neural Regen Res 11(5):842-845.*

Abstract

The aim of the present study was to evaluate whether tissue levels of vitamin B complex and vitamin B_{12} were altered after crush-induced peripheral nerve injury in an experimental rat model. A total of 80 male Wistar rats were randomized into one control ($n = 8$) and six study groups (1, 6, 12, 24 hours, 3, and 7 days after experimental nerve injury; $n = 12$ for each group). Crush-induced peripheral nerve injury was performed on the sciatic nerves of rats in six study groups. Tissue samples from the sites of peripheral nerve injury were obtained at 1, 6, 12, 24 hours, 3 and 7 days after experimental nerve injury. Enzyme-linked immunosorbent assay results showed that tissue levels of vitamin B complex and vitamin B_{12} in the injured sciatic nerve were significantly greater at 1 and 12 hours after experimental nerve injury, while they were significantly lower at 7 days than in control group. Tissue level of vitamin B_{12} in the injured sciatic nerve was significantly lower at 1, 6, 12 and 24 hours than in the control group. These results suggest that tissue levels of vitamin B complex and vitamin B_{12} vary with progression of crush-induced peripheral nerve injury, and supplementation of these vitamins in the acute period may be beneficial for acceleration of nerve regeneration.

***Correspondence to:**
Idiris Altun, M.D.,
idrisaltun46@hotmail.com.

orcid:
0000-0003-4263-766X
(Idiris Altun)

doi: *10.4103/1673-5374.177150*
http:*//www.nrronline.org/*

Accepted: *2015-12-22*

Key Words: *nerve regeneration; sciatic nerve injury; vitamin B complex; vitamin B_{12}; neural regeneration*

Introduction

Following peripheral nerve injury, a complex reparative process starts to eliminate the damage and restore the structure and function. Unlike cellular repair in other areas in human body, peripheral nerve injury does not involve mitosis and cellular proliferation (Burnett and Zager, 2004). With advances in molecular biology, increasing attention has been paid to the process of peripheral nerve regeneration. The response of peripheral nerve to injury extends beyond the site of damage but involves neural cells in the spinal cord and ganglia. Schwann cells, macrophages, inflammatory cells, and neurotrophic factors are involved in this complex cascade (Rishal and Fainzilber, 2010). The injured peripheral nerve attempts to compensate the lost functions by strengthening and reprogramming the uninjured pathways (Burnett and Zager, 2004). The whole regeneration and repair process after peripheral nerve injury has not been completely understood. The peripheral nerves are capable of regeneration after injury on their own due to the activation of their intrinsic growth capacity (Rishal and Fainzilber, 2010). The sciatic nerve is commonly used for studying peripheral nerve regeneration since it consists of both sensory and motor neurons (Bridge et al., 1994; Hobbenaghi et al., 2012).

Vitamin B complex helps to alleviate degeneration in the nervous system and vitamin B_1 (thiamine), vitamin B_6 (pyridoxine) in combination with vitamin B_{12} are clinically administered (Corinne et al., 2009). These vitamins, in particular vitamin B_{12}, exhibit important roles in various biological events to maintain normal neural functions (Corinne et al., 2009; Hobbenaghi et al., 2012). Application of vitamin B complex or vitamin B_{12} has been shown to increase the number of Schwann cells and myelinated nerve fibers and the diameter of axons, and thereby promote the regeneration of myelinated nerve fibers and the proliferation of Schwann cells (Lopatina et al., 2011). Treatment with vitamin B complex or vitamin B_{12} increases the expression of brain-derived neurotrophic factor (BDNF) in the injured sciatic nerves at both mRNA and protein levels, but it does not affect on the expression of glial cell-derived neurotrophic factor, neurotrophin-3, and interleukin-6; in addition, vitamin B complex and vitamin B_{12} have been shown to promote the regeneration and functional recovery of injured sciatic nerves through increasing BDNF expression (Sun et al., 2012). Supplementation of vitamin B_{12} may enhance peripheral nerve regeneration (Watanabe, 1994). High dose vitamin B_{12} has been shown to have the potential to treat peripheral nerve injury (Okada and Tanaka, 2010).

The aim of the current study was to evaluate whether vitamin B complex and vitamin B_{12} levels were affected after crush-induced peripheral nerve injury in an experimental rat model. To the best of our knowledge, no articles have been published on this topic and our study is the first to investigate the levels of vitamin B complex in injured peripheral nerve tissue in animal models.

Materials and Methods

Animals

This study was approved by Institutional Animal Care and

Use Committee of Kahramanmaras Sutcu Imam University Medical Faculty. Eighty Wistar rats obtained from Experimental Animal Room of Kahramanmaras Sutcu Imam University Medical Faculty were included in this study. They were kept at constant temperature (20–22°C) and humidity (50–60%) in 12-hour dark/light cycle and given with *ad libitum* food and water. Procedures were carried out in accordance with the guidelines of the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). All efforts were made to minimize the number of animals used and their suffering.

Surgical procedures

Eighty Wistar rats, weighing 200–250 g were randomly divided into one control group ($n = 8$) and six study (1st, 6th, 12th hours, 3rd, 7th days) groups after experimental nerve injury; $n = 12$ for each group). Surgical interventions were carried out under anesthesia with a premixed solution containing 15 mg/kg xylazine and 100 mg/kg ketamine. Rat models of peripheral nerve injury were established as described by Renno et al. (2013). The right sciatic nerve was exposed at the mid-thigh level and crushed at the location 1 cm from the sciatic notch for 60 seconds with a mosquito forceps. The skin wound was closed with fine sutures. In the control group, the right sciatic nerves identifiably underwent anesthesia and the incisions were closed without any surgical intervention on sciatic nerve. In the control group, immediately after the sciatic nerve was exposed, sciatic nerve tissue was harvested for biopsy. All procedures were implemented by the same surgeon (Idiris Altun) using the same equipment. Nerve injury may depend on the length of time of crush insult. During our earlier trials, we tried different time frames ranging from 10 to 120 seconds. The 60 second compression injury in sciatic nerve showed all the motor and sensory symptoms of a typical crushed nerve reported previously by many investigators (Bridge et al., 1994; Sun et al., 2012; Hobbenaghi et al., 2013; Renno et al., 2013). İn the current six study groups, sciatic nerve tissues were harvested at different time points (1, 6, 12, 12 hours, 3 and 7 days after crush injury) for biopsies.

Preparation of nerve tissue homogenates

Nerve tissue samples were homogenized in two volumes (w/v) of the 1.15% ice-cold KCl solution, using a Heidolph 50110 R2R0 homogenizer (Schwabach, Germany). The homogenate was centrifuged in a Sorvall RC-2B (Minneapolis, MN, USA) at 39, 880 × *g* for 30 minutes at 4°C and the supernatant was collected for biochemical assays.

ELISA test procedure

Vitamin B_{12} levels were determined according to the test procedure of Ridascreen Fast Vitamin B_{12} ELISA (R-Biopharm A.G. Kit, Art No: R2102 Darmstad, Germany) and results were evaluated using an ELISA reader (DAS). A sufficient number of wells were inserted into the microwell holder for all standards and samples to be run. The standard and sample positions were recorded. Absorbance at 450 nm was measured.

Detection of vitamin B complex and vitamin B_{12} levels

Vitamin B complex and vitamin B_{12} levels in tissue homogenate were determined according to the test procedure of Eagle Biosciences Vitamin B_{12} ELISA (Catalog Number B1206-K01 Nashua, New Hampshire, USA) and results were evaluated using an ELISA reader. The ELISA assay was performed as above.

Statistical analysis

Data analysis was performed *via* IBM Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA) and normal distribution of quantitative data was checked with the Kolmogorov-Smirnov test. Parametric tests were applied to normally distributed data, while non-parametric tests were administered for normally distributed data. One-way analysis of variance was used for comparison of groups with independent continuous variables and Duncan's multiple range test was used for comparison tests. Continuous data were expressed as the mean ± SD or median [minimum-maximum] and a level of $P < 0.05$ was considered statistically significant.

Results

Data collected in this study were found to have a normal distribution ($P = 0.857$ for vitamin B complex; $P = 0.926$ for vitamin B_{12}). One-way analysis of variance showed that there was significant difference in vitamin B complex level between groups ($P = 0.001$) and homogeneous subsets were designated according to the results of Duncan's multiple range tests (**Table 1**). Biopsies of sciatic nerve taken at 1, 12 hours and 7 days after experimental nerve injury showed the most significant differences as compared to the control group. Higher level of vitamin B complex was detected in sciatic nerve taken at 1 and 2 hours after experimental nerve and lower level of vitamin B complex in sciatic nerve taken at 7 days ($P < 0.05$). The mean levels of vitamin B complex

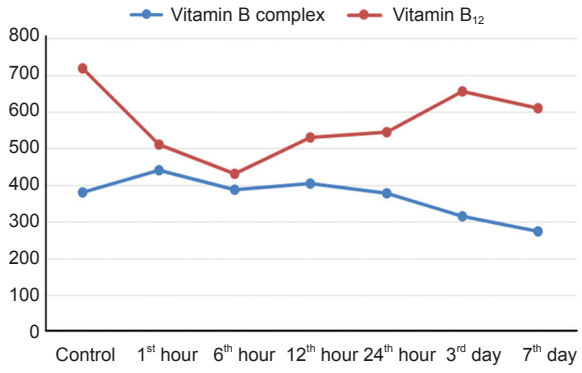

Figure 1 Mean vitamin B complex and vitamin B_{12} levels in the injured sciatic nerve of rats.

Compared to the control group, higher level of vitamin B complex was detected in sciatic nerve taken at 1 and 2 hours after injury and lower level of vitamin B_{12} in the sciatic nerve taken at 7 days after injury ($P < 0.05$). One-way analysis of variance followed by Duncan's multiple range tests was used for comparisons.

Table 1 Duncan's multiple range test results for vitamin B complex levels in study and control groups

		Homogeneous subsets		
Group	mean±SD	1	2	3
7th day	274.50±80.85	*		
3rd day	315.67±100.70	*	*	
24th hour	379.58±98.44		*	*
Control	381.00±87.51		*	*
6th hour	389.83±115.09		*	*
12th hour	405.58±88.02			*
1st hour	442.50±82.56			*

SD: Standard deviation. *: Statistically significant.

Table 2 Duncan's multiple range test results for vitamin B_{12} levels in study and control groups

		Homogeneous subsets			
Group	mean±SD	1	2	3	4
1st hour	511.83±98.71	*	*		
6th hour	431.83±94.00	*			
12th hour	531.67±107.95	*	*	*	
24th hour	547.00±175.00	*	*	*	
7th day	611.50±225.46		*	*	*
3rd day	656.83±142.47			*	*
Control	718.75±199.69				*

Significant differences were detected between control and 1st hour, 12th hour or 7th day groups ($P < 0.05$). *: Statistically significant. SD: Standard deviation.

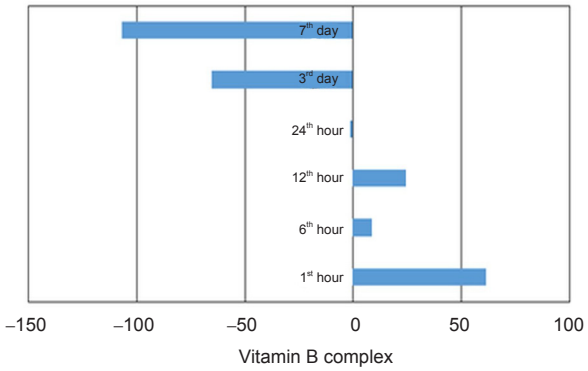

Figure 2 Deviation of vitamin B complex level in the study groups from that in the control group.
Data were expressed as the mean ± standard deviation.

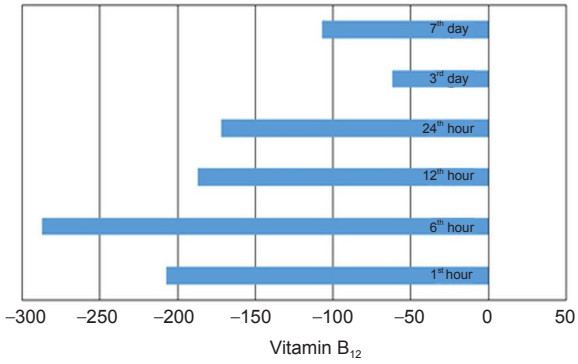

Figure 3 Deviation of vitamin B_{12} level in the study groups from that in the control group.

in the control and study groups are shown in **Figure 1**. Deviations of the mean values of the study groups from the control group are shown in **Figures 2** and **3**. One-way analysis of variance results showed that there was significant difference in vitamin B_{12} level between groups ($P = 0.001$) and four homogeneous subsets were designated according to the results of Duncan's multiple range tests (**Table 2**). Significant differences were detected between control and 1st hour, 12th hour and 7th day groups. In 1-hour, 12-hour and 7-day study groups, the mean levels of vitamin B_{12} were significantly lower than that in the control group ($P < 0.05$).

Discussion

In the current study, we aimed to investigate whether vitamin B complex and vitamin B_{12} levels in rat sciatic nerve tissue were affected after experimental nerve injury. Our results indicate that peripheral nerve injury is accompanied by various levels of vitamin B complex and vitamin B_{12}.

B vitamins such as thiamine (B_1), pyridoxine (B_6) and cyanocobalamin (B_{12}) have antinociceptive effects in experimental animals with acute and chronic pain linked with neuronal injury (Wang et al., 2005; Caram-Salas et al., 2006; Yu et al., 2014). Vitamins B_6 and B_{12} have been shown to counteract the deleterious effects of ischemia on neurons (Kaneda et al., 1997; Hung et al., 2009). These vitamins play important roles in nutrition, nerve conduction, axonal transport, synthesis of neurotransmitters and excitation *via* cyclic guanosine monophosphate (c-GMP) signaling pathway (Wang et al., 2005). Therefore, reconstructive activity of vitamin B complex on degenerative nerves may facilitate the recovery of nerve function after peripheral nerve injury. Resolution of peripheral nerve injury may benefit from a multinutrient treatment approach since vitamin B complex are necessary for optimum functioning of the nervous system (Maladkar et al., 2014). Unavailability of these vitamins may decrease the enzymatic activity of some enzymes and result in neurotoxicity.

Vitamin B_{12} is a scavenger of the reactive oxygen species and has a neuroprotective function owing to its anti-apoptotic and anti-necrotic effects on neurons (Sun et al., 2012; Liao et al., 2013). Vitamin B_{12} increases the regeneration of axons and the metabolic pathway of vitamin B_{12} is closely related to neuronal survival and repair after injury (Dominguez et al., 2012; Sun et al., 2012; Hobbenaghi et al., 2013). Improvement of the delivery system for B vitamins may provide both new eras for treatment of nerve injuries and yield new insights for understanding the pathophysiology. These vitamins may have synergistic effects that cause production of endogenous neurotropic factors, which enhance peripheral nerve repair (Sun et al., 2012).

Hopefully, investigations into this area will undoubtedly yield information that will lead to surgical and therapeutic advances for treatment of peripheral nerve injury such as the use of neurotrophic factors to stimulate growth and direct growing axons to their proper target organ. Perhaps this additional understanding will also help explain why the central nervous system has not developed such a restorative process and illuminate some possible strategies for simulating the peripheral nerve regenerative microenvironment in the brain and spinal cord. Efforts must be directed for prevention of deficiency of these vitamins and the value of vitamin B supplementation in cases of peripheral nerve injury needs to be investigated in further trials.

The limitations of the current study include lack of histopathological examination and disadvantages of experimental design. Moreover, biochemical results are prone to be influenced by technical and environmental factors. Due to these factors, associations must be made with caution.

To conclude, we demonstrated that tissue levels of vitamin B complex and vitamin B_{12} vary with progression of peripheral nerve injury. Supplementation of vitamin B complex in the acute period of peripheral nerve injury deserves to be considered as a treatment option, which may be useful for acceleration of nerve regeneration.

Author contributions: *İA designed the study, performed surgery and wrote the paper. EBK provided assistance in technique application. Both of these two authors approved the final version of the paper.*
Conflicts of interest: *None declared.*
Plagiarism check: *This paper was screened twice using Cross-Check to verify originality before publication.*
Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K (1994) Nerve crush injuries a model for axonotmesis. Exp Neurol 127:284-290.
Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. Neurosurg Focus 16:E1.
Caram-Salas NL, Reyes-García G, Medina-Santillán R, Granados-Soto V (2006) Thiamine and cyanocobalamin relieve neuropathic pain in rats: synergy with dexamethasone. Pharmacology 77:53-62.
Corinne G, Mizisin LM, Austin N (2009) B vitamins alleviate indices of neuropathic pain in diabeticrats. Eur J Pharmacol 612:41-47.
Dominguez JC, Ng AR, Damian LF (2012) A prospective, open label, 24-week trial of methylcobalamin in the treatment of diabetic polyneuropathy. J Diabetes Mellitus 2:408-412.
Hobbenaghi R, Javanbakht J, Hosseini E, Mohammadi S, Rajabian M, Moayeri P, Aghamohammad Hassan M (2013) Neuropathological and neuroprotective features of vitamin B12 on the dorsal spinal ganglion of rats after the experimental crush of sciatic nerve: an experimental study. Diagn Pathol 8:123.
Hung KL, Wang CC, Huang CY, Wang SJ (2009) Cyanocobalamin, vitamin B12, depresses glutamate release through inhibition of voltage-dependent Ca^{2+} influx in rat cerebrocortical nerve terminals (synaptosomes). Eur J Pharmacol 602:230-237.
Kaneda K, Kikuchi M, Kashii S, Honda Y, Maeda T, Kaneko S, Akaike A (1997) Effects of B vitamins on glutamate-induced neurotoxicity in retinal cultures. Eur J Pharmacol 322:259-264.
Liao WC, Wang YJ, Huang MC, Tseng GF (2013) Methylcobalamin facilitates collateral sprouting of donor axons and innervation of recipient muscle in end-to-side neurorrhaphy in rats. PLoS One 8:e76302.
Lopatina T, Kalinina N, Karagyaur M, Stambolsky D, Rubina K, Revischin A, Pavlova G, Parfyonova Y, Tkachuk V (2011) Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. PLoS One 6:e17899.
Maladkar M, Tekchandani C, Dave U (2014) Post-marketing surveillance of fixed dose combination of methylcobalamine, alpha lipoic acid, folic acid, biotin, benfotiamine & vitamin B6-nutripathy for the management of peripheral neuropathy. J Diabetes Mellitus 4:124-132.
Okada K, Tanaka H (2010) Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Exp Neurol 222:191-203.
Renno WM, Al-Maghrebi M, Alshammari A, George P (2013) (-)-Epigallocatechin-3-gallate (EGCG) attenuates peripheral nerve degeneration in rat sciatic nerve crush injury. Neurochem Int 62:221-231.
Rishal I, Fainzilber M (2010) Retrograde signaling in axonal regeneration. Exp Neurol 223:5-10.
Sun H, Yang T, Li Q, Zhu Z, Wang L, Bai G, Li D, Li Q, Wang W (2012) Dexamethasone and vitamin B(12) synergistically promote peripheral nerve regeneration in rats by upregulating the expression of brain-derived neurotrophic factor. Arch Med Sci 8:924-930.
Wang ZB, Gan Q, Rupert RL, Zeng YM, Song XJ (2005) Thiamine, pyridoxine, cyanocobalamin and their combination inhibit thermal, but not mechanical hyperalgesia in rats with primary sensory neuron injury. Pain 114:266-277.
Watanabe TE (1994) Ultrahigh dose methylcobalamin prometes nerve regeneration in experimental acrylamid neuropathy. J Neurol Sci 12:140-143.
Yu CZ, Liu YP, Liu S, Yan M, Hu SJ, Song XJ (2014) Systematic administration of B vitamins attenuates neuropathic hyperalgesia and reduces spinal neuron injury following temporary spinal cord ischaemia in rats. Eur J Pain 18:76-85.

Copyedited by Cakmak H, Drummond PD, Fei Y, Li Y, Li CH, Song LP, Zhao M