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The Contribution of Thiamine, Pyridoxine and Cyanocobalamine Combination on Nerve Regeneration in Rats with Experimentally Induced Sciatic Injury

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Abstract: The aim of this study was to investigate the role of thiamine, pyridoxine and cyanocobalamine combination on nerve regeneration after experimentally induced crush injury in thirty adult, male wistar rats. Sciatic function index and Toe spreading reflex and nerve conduction studies on exposed sciatic nerve were carried out before sciatic crush injury and after three weeks of treatment. The experiment group received thiamine (33 mg kg^{-1}), pyridoxine (33 mg kg^{-1}) and cyanocobalamine (0.5 mg kg^{-1}) injections intramuscularly for 3 weeks and the control group received equal volumes of saline injections. Toe-spreading reflex were better in the experiment group than the controls after treatment ($p< 0.05$). There was no significant difference in regard to DL, DCMAP, PCMAP and NCV between both groups ($p<0.05$). In conclusion the beneficial effect of thiamine, pyridoxine and cyanocobalamine combination was not found in regard to the electrophysiological parameters measured. However clinical improvement was superior in the experiment group than the control group.

Key words: Sciatic nerve, crush, vitamin B, electrophysiology, rat, thiamine

INTRODUCTION

Complete severing of the peripheral nerves is required for microsurgical repair and the main problem is related to the formation of scar tissue and neuroma in the repaired site (Hoerlein, 1965; Besalti *et al*., 2002). However many attempts are focused in improvement or acceleration of nerve regeneration after incomplete injury or as ancillary treatments in addition to surgical repair such as physical therapy, or drugs including many tropic factors (Hoerlein, 1965; Besalti *et al*., 2002; Makwana and Raivich, 2005).

Thiamine (B_1), pyridoxine (B_6) and cyanocobalamine (B_{12}) combination is used for their role in axonal transport, excitability of neurons or synthesis of neurotransmitters (Wang *et al*., 2005). B vitamins have an important role in peripheral and central nervous system (Becker *et al*., 1990). As the dosage range is very large, they are frequently administered in a widely varying number of disorders of the nervous system such as neuritis, polyneuropathy, ischialgia, lumbalgia, peripheral nerve paresis, carpal tunnel syndromes, traumatic disorders of the central and peripheral nervous system (Wang *et al*., 2005; Becker *et al*., 1990). Some authors suggest using vitamin B in cases having deficiency and consider administration of vitamins as a supportive measure for the casual therapy as superfluous and without additional benefit and the others reject such therapy completely (Becker *et al*., 1990).

Vitamin deficiency syndromes can lead to the disintegration of the myelin sheath and to axonal damage in the central as well as in the peripheral nervous system and can be relieved by appropriate therapeutic measures (Chaney, 1992). This finding leads to the recommendation that B group vitamins can also be administered in cases with lesions of peripheral nerves. However the beneficial effects of using B vitamins as a supportive measure remain controversial in both clinical and experimental trial. The long term and excessive administration of pyridoxine causes toxic damage to the nervous system (Dalton and Dalton, 1987; Krinke *et al*., 1985).

The purpose of the study was to investigate the contribution of vitamins B_1, B_6 and B_{12} combination on nerve regeneration in an experimentally induced nerve injury. Considering the excessive usage of B vitamins to aid the regeneration of peripheral or central nervous system injury in the field of veterinary practice, this study was designed.

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MATERIALS AND METHODS

The procedures employed for this study were approved by the ethic committee of the Faculty of Veterinary Medicine of Ankara University. The experiment was carried out on 30 adult male wistar rats weighing 220-270 g at the beginning of the experiment. The rats were housed in groups of five in stainless steel cages (40×50×30 cm) with soft bedding and free access to food and water under a 12 h day/12 h night cycle.

Anesthesia and crushing procedures: Anesthesia was induced by ketamin hydrochloride (60 mg kg^{-1}) and xylasine hydrochlorure (9 mg kg^{-1}) intraperitoneally. The left leg was prepared for aseptic surgery. The left common sciatic nerve of each rat was exposed at the thigh between sciatic notch and bifurcation to the tibial and peroneal nerve. The area to be crushed was marked by knotting propylene sutures one cm proximally to the bifurcation. The nerve was crushed with a smooth bulldog clamp for 3 min and the operation area was closed in a routine manner.

Clinical evaluation

Walking track analysis: Walking tracks were obtained as previously described using an 8.2×42 cm corridor open at one end to a darkened compartment. The hind feet were dipped in x-ray developer and the rats were allowed to walk down the corridor on a sheet of undeveloped x-ray film. Walking procedure was repeated in case of clear print marks were not obtained. Walking tracks were measured by a manual caliper. Paired measurement of Print Length (PL), Toe Spread (TS-first to fifth Toe), Intermediary Toe spread (IT-second to fourth Toe) were recorded for both normal (NPL, NTS, NIT) and experimental (EPL, ETS, EIT) feet (Fig. 1). For estimating Sciatic Function Index (SFI), the reported formula was used (Brain *et al.*, 1989). SFI was recorded and estimated before crush injury, at the 24th h, 7th day and 21st day.

$$SFI = -38.3(EPL-NPL/NPL)+109.5(ETS-NTS/NTS)+13.3(EIT-NIT/NIT)-8.8$$

Toe-spreading reflex was evaluated while the rat was held up from its tail as normal, medium, slight and completely depressed.

Medelec Synergy (Oxford instrument) 5 channel EMG/EP machine was used for the electrophysiological studies. The stimulating electrodes were inserted 1cm below the crush point and 1 cm above the crush point as active electrode inserted epineurally and reference electrode inserted about 0.3 cm laterally, subfascially. The

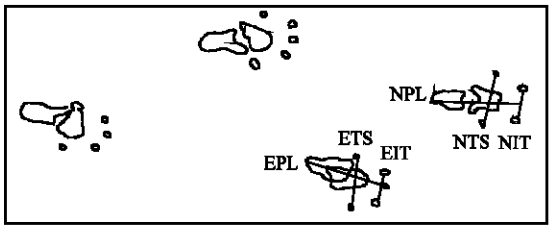

Fig. 1: Foot prints of the rats hind legs. Paired measurement of Print Length (PL), Toe Spread (TS-first to fifth Toe), Intermediary Toe spread (IT-second to fourth Toe) were recorded for both normal (NPL, NTS, NIT) and experimental (EPL, ETS, EIT) feets

recording electrode was inserted subcutaneously over the plantar muscle at the midpoint of its entire length and referred to its tendon subcutaneously. Both stimulation and recording procedures were performed with monopolar needle electrodes. The recording procedures were carried out as stimulation from distal and proximal crush points and recording from plantar muscle. Frequency limits for recording were 10 Hz-2 KHz, sweep speed was 10 msecs. A rectangular, 0.1 ms duration stimulus at supramaximal intensity was used and at least 3 consecutive, repeatable Compound Muscle Action Potentials (CMAP) were adjusted. The amplitude was measured as peak to peak and the latency was measured as onset latency and NCV was estimated as the distance division to the difference between proximal and distal latency. Electrophysiological studies were performed at pre-crush, after crush and 21st day.

The animals were allocated into two groups of 15 rats, one of which was treated with thiamine (33.3 mg kg^{-1}), pyridoxine (33.3 mg kg^{-1}) and cyanocobalamine (0.5 mg kg^{-1}) over the predetermined period and the control group received saline. The B vitamins administered in this study are contained in a standard combination drug (Neurogriseovit) in the following composition:

- Vitamin B1 (thiamine hydrochloride): 33.3 mg mL^{-1}
- Vitamin B6 (pyridoxine hydrochloride): 33.3 mg mL^{-1}
- Vitamin B12: 1 mg mL^{-1}
- Lidocaine hydrochloride: 5 mg mL^{-1}

The medication started on the second day and continued until the 21st day. At the end of this period the animals were anesthetized and their sciatic nerves were exposed and electrophysiological control was performed as mentioned for pre-injury.

Statistical analysis: Distal motor latency, DCMAP, PCMAP and NCV were analyzed with repeated measurement of variance analysis. The mean difference of intervals and comparison of groups were analyzed with Duncan's multiple rang test and Bonferroni test. For all tests $p<0.05$ was accepted as significant.

RESULTS

Toe spread analysis was found totally depressed at the 24th h in all cases of both control and experimental groups and there were no differences between the groups. The same results were seen at the 7th day. However, the results obtained at the 21st day were different and favorable results were observed in experimental group. Normal Toe-spreading reflex in 3 and median Toe-spreading reflex in 11 cases were observed in experiment group, while slight Toe-spreading reflex in 8 and median Toe-spreading reflex in 7 were observed in control group at the 21st day.

Sciatic function index values that were estimated before crush injury are found as -17.73±6.17 and -78.16±10.25, -61.88±12.34 and -15.52±5.22 at the 24th h, 7th day and 21st day consequently in evaluated cases in regard to intervals and without taking the groups into account.

In experiment group, SFI was estimated as-16.91±5.22, -78.60±13.21, -63.49±12.87 and -15.55±2.16 for different intervals mentioned above consequently. In control group, SFI was -18.55±7.09, -77.73±6.53, -60.28±12.00 and -15.48±7.19 for different intervals mentioned above consequently. Mean value for time was found significant according to intervals. However, the differences between mean values of groups and group-intervals interaction were not significant.

The electrophysiological pre-crush values for control groups were 1.93 ms, 48.69 m sec^{-1}, 10.02 mV, 10.62 mV; at the 21st day values for control groups were 2.57 ms, 36.61 m sec^{-1}, 2.34 mV and 2.60 mV as DML, MNCV, pCMAP and dCMAP. The values recorded at the post-crush were 2.21 ms, 0.04 mV, 10.81 mV as DML, pCMAP, dCMAP.

The values for experiment groups were 2.18 ms, 51.47 m sec^{-1}, 9.11 mV, 10.14 mV; at the 21st day values for experiment groups were 2.59 ms, 34.55 m s^{-1}, 2.03 mV, 2.36 mV as DML, MNCV, pCMAP and dCMAP. The values recorded at the post crushing were 2.51 ms, 0.24 mV, 10.33 mV as DML, pCMAP, dCMAP. The electrophysiological values were introduced in Fig. 2-5.

Normative electrophysiological values were 2.06±0.25 ms, 10.38±2.95 mV, 9.57±3.04 mV, 50.08±5.91 m s^{-1} for DL, dCMAP, pCMAP and NCV,

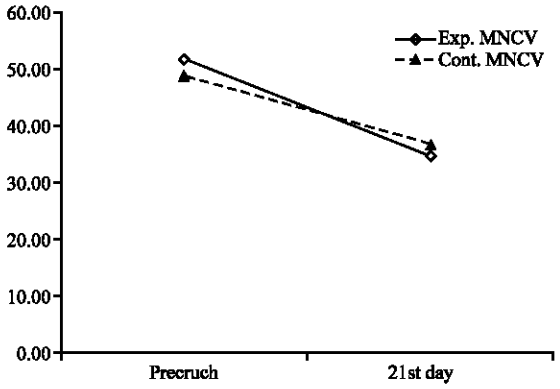

Fig. 2: Comparison of MNCV pre-crush and 21st day values in experiment and control groups ($p>0.05$)

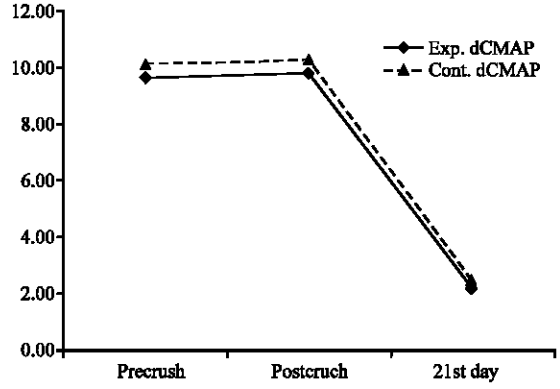

Fig. 3: Comparison of dCMAP pre-crush, post-crush and 21st day values in experiment and control groups ($p>0.05$)

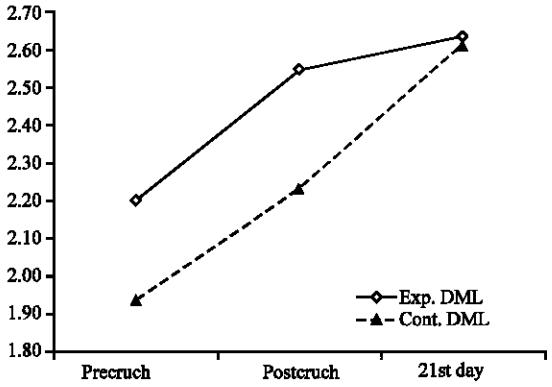

Fig. 4: Comparison of Distal Motor Latency (DML) pre-crush, post-crush and 21st day values in experiment and control groups ($p>0.05$)

respectively in 30 rats. In the experiment group, the same values were 2.18±0.22 ms, 10.14±2.70 mV, 9.11±2.52 mV,

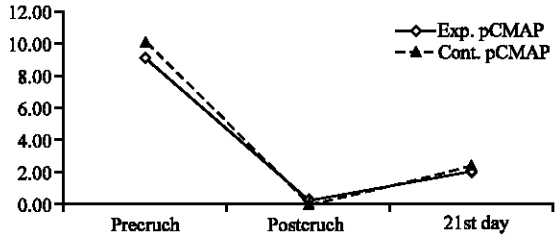

Fig. 5: Comparison of pCMAP pre-crush, post-crush and 21st day values in experiment and control groups ($p>0.05$)

51.47±6.78 m s^{-1}, however in the control group they were 1.93±0.21 ms, 10.62±3.27 mV, 10.02±3.50 mV and 48.69±4.72 m s^{-1}, respectively at the 21st day. There was no significant difference in regard to DL, DCMAP, PCMAP and NCV between both groups ($p<0.05$). When the normative and post treatment values were compared, the differences were found significant ($p<0.05$).

DISCUSSION

The effects of vitamin B on lesioned peripheral nerves have been investigated by different authors. B group vitamins have a regenerating and accelerating effect (Nechai, 1968; Hasegawa *et al*., 1973), methylcobalamin may have inhibiting processes during wallerian degeneration and stimulating protein synthesis of the Schwann cells during regeneration (Mikhailov *et al*., 1983; Yamatsu *et al*., 1976a,b) and following the repair of facial nerve, has a favorable effect (Matsumoto *et al*., 1981). In conclusion it was suggested that the positive effects of B vitamins on nerve regeneration are plausible possibilities. Vitamin B1, B6 and B12 combination usage was also experienced in sensory nerve injury and it has reached a favorable conclusion (Becker *et al*., 1990). Recently, Wang *et al*. (2005) demonstrated the effects of vitamins B on pain and hyperalgesia following primary sensory neuron injury and suggest the possible clinical utility of B vitamins in the treatment of neuropathic painful conditions following injury, inflammation, degeneration or other disorders in the nervous system. The favorable effects on the nerve dysfunction associated with diabetes and neurite outgrowth in acryl amide neuropathy model was also reported (Fujii *et al*., 1996; Fukuda *et al*., 1979). Vitamin B6 was also suggested for carpal tunnel syndrome in men (Bender, 1999; Keniston *et al*., 1997). However motor and sensory neuropathy secondary to excessive pyridoxine ingestion was reported in a clinical case (Foca, 1985).

To the author's knowledge, the contribution of B Vitamins was not studied in regard to clinical and electrophysiological examination. When the clinical examination's results of the study presented here were analyzed, discrepancy between Toe spreading test and SFI appear, but gradual improvement is remarkable in all rats after crushing period. In a rat sciatic nerve crush injury model, significant differences between control and crush SFI values after two weeks was not observed (Hare *et al*., 1992). Even though the crush procedure was different in our study (crush with bulldog clamp for 3 min), there was not significant differences in all intervals between both groups.

Compound muscle action potential amplitude is the vectoral sum of action potentials occurring as a result of the contraction of all muscle fibers after the stimulation of the nerve. As the muscle fibers become thicker and increase in number, the CMAP amplitude is expected to be greater after the regeneration (Kimura, 1989). In the post-crush recordings, decreasing pCMAP near to the baseline represent the complete conduction block, because of the axonal loss and demyelization and for that reason NCV could not estimated at this stage. Unchanged dCMAP after crushing is expected cases because the myelin sheath and axons at the distal part are still intact. At the 21st day dCMAP, pCMAP and MNCV were remained five times lower level when they compared their pre-crush values. The absence of significant differences between groups in regard to these values represents no superiority of each to other. The beneficial effects of B vitamins were not observed according electrophysiological evaluation. However regeneration has occurred in both groups by the time.

The discrepancy between SFI, electrophysiological studies and Toe spreading reflex can be explained with the qualitative property of Toe spreading rather than others. Another conclusion can be inferred from this study that; even though even though decreasing amplitude about its 20% of pre-crush values clinical score can be at the satisfactory level.

CONCLUSION

The beneficial effects of B vitamins were not determined with this study except for the Toe spread test. However, further studies should be done especially in transected and repaired nerve injury model.

REFERENCES

Bain, J.R., S.E. Mackinnon and D.A. Hunter, 1989. Functional evaluation of complete sciatic, peroneal and posterior tibial nerve lesions in the rat. Plast Reconstr. Surg., 83: 129.

Becker, K.W., E.W. Kienecker and P. Dick, 1990. A contribution to the scientific assessment of degenerative and regenerative processes in peripheral nerve fibers following axonotmesis under the systemic administration of vitamins B1, B6 and B12-light and electron microscopy findings in the saphenous nerve of the rabbit. Neuroshirurgia, 33: 113-121.

Bender, D.A., 1999. Non-nutritional uses of vitamin B6. Br. J. Nutr., 81: 7-20.

Besalti, O., A. Ozak, F.E. Ozgencil, S. Bilgihan and F. Akin, 2002. Comparison of lyophilized duramater and autogenous omental wrappings of grafting sites in experimentally induced facial nerve injury. Part I Clinical and gross evaluations. Turk. J. Vet. Anim. Sci., 26: 273-278.

Chaney, S.G., 1992. Textbook of Biochemistry with Clinical Correlations. Thomas, MD., (Ed.), Wiley-Liss Publications, New York

Dalton, K. and M.J.T. Dalton, 1987. Characteristics of pyridoxine overdose neuropathy syndrome. Act Neurol. Scand., 76: 8-11.

Foca, F.J., 1985. Motor and sensory neuropathy secondary to excessive pyridoxine ingestion. Arch Phys. Med. Rehabil., 66: 634-636.

Fukuda, N., H. Ikeda, A. Shino, H. Iwatsuka, Y. Nagawa, 1979. Effect of vitamin B1, B6 and B12 on the sciatic nerve in alloxan diabetic rats. Vitamins, 53: 513-521.

Fujii, A., H. Matsumoto and H. Yamamoto, 1996. Effect of vitamin B complex on neurotransmission and neurite outgrowth. Gen. Pharmac., 27: 995-1000.

Hare, G.M.T., P.J. Evans, S.E. Mackinnon, T.J. Best, J.R. Bain, J.P. Szalai and R.T. Hunter, 1992. Walking track analysis: A long-term assessment of peripheral nerve recovery. Plast. Reconstr. Surg., 89: 251-258.

Hasegawa, K., S. Homma and K. Kanda, 1973. Effects of vitamin B1, B6 and B12 complex on the regeneration of the peripheral nerve and muscle receptor in cats. Folia. Pharm. Jap., 69: 483-497.

Hoerlein, B.F., 1965. Canine Neurology Diagnosis and Treatment. WB Saunders Company, Philadelphia.

Keniston, R.C., P.A. Nathan, J.E. Leknem and R.S. Lockwood, 1997. Vitamin B6, vitamin C and carpal tunnel syndrome. JOEM, 39: 949-959.

Kimura, J., 1989. Electrodiagnosis in Disease of Nerve and Muscle: Principles and Practice (2nd Edn.), Philadelphia, F.A. Davis Company, pp: 78-97.

Krinke, G., D.C. Naylor and V. Skorpil, 1985. Pyridoxine megavitaminosis: An analysis of the early changes induced with massive doses of vitamin B6 in rat primary sensory neurons. J. Neuropath. Exp. Neurol., 44: 117-129.

Makwana, M. and G. Raivich, 2005. Molecular mechanisms in succesful peripheral regeneration. FEBS. J., 272: 2628-2638.

Mikhailov, V.V., V.V. Mikhailov and V.M. Avakumov, 1983. On the mechanism of the effect of methylcobalamin on processes of the recovery of the neuromuscular appratus in mechanical and toxin-induced denervation. Farmakol. Toksikol., 46: 9-12.

Matsumoto, Y., N. Yanagihara and H. Okamura, 1981. Administration of methylcobalamin after surgical repair of the fasial nerve. Pract. Otol., 74: 2301-2307.

Nechai, E.E., 1968. The effect of fasting on nerve regeneration in combined injures. Vrach. Delo., 2: 70-74.

Wang, Z.B., Q. Gan, R.L. Rupert, Y.M. Zeng and X.J. Song, 2005. Thiamine, pyridoxine, cyanocobalamin and their combination inhibit thermal, but not mechanical hyperalgesia in rats with primary sensory neuron Injury. Pain, 114: 266-277.

Yamatsu, K., T. Kaneko, A. Kitahara and I. Ohkawa, 1976. Pharmacological studies on degeneration and regeneration of peripheral nerves. I. Effects of methylcobalamin and cobamide on EMG patterns and weight loss od denervated muscles following crush of the sciatic nerve in rats. Folia. Pharmacol. Jap., 72: 259-268.

Yamatsu, K., Z. Yamanishi, T. Kaneko and I. Ohkawa, 1976. Pharmacological studies on degeneration and regeneration of peripheral nerves. II. Effects of methylcobalamin on mitosis of Schwann cells and incorporation of radioactive leucine intoprotein fraction of crushed sciatic nerve in rats. Folia. Pharmacol. Jap., 72: 259-268.