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The Effect of Vitamins B on Myelination of Injured Peripheral Nerve in Rat

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Abstract: ***Introduction: Neuropathy is one of the common neurological problem which is cause by many factors, one of these factors is trauma. Traumatic neuropathy affects myelin sheath with or without nerve fiber. Demyelination includes disintegration of myelin sheath and myelin nucleus. Many medications have been used to treat traumatic neuropathy. One of these medications is vitamin B. Aim of the study: Effect of vitamins B on myelination of degenerative traumatic neuropathy. Material and Method: Forty albino rats were used in this study. All the animals exposed to crush injury of the sciatic nerve. These experimental animals were divided into 4 groups. Three of them received one type of vitamins B including vitamin B1, B6, B12 besides, the forth group received placebo. After 6weeks of experiments, crushed area of sciatic nerve was studies histologically and immunohistochemically. Result: The group of animals, Which exposed to crush injury of sciatic nerve and treated with vitamin B12 revealed best regenerative process more than that which received B1 which in turn elicited better result than that group received vitamin B6. Discussion: Vitamin B12 has a positive effects on myelination of nerve fiber, therefore it processed better regenerative reaction than that treated with vitamins B1 and B6.***

1. Introduction

Peripheral nervous system is a complementary to the central nervous system. It consists of peripheral nerve which could be classified as cranial, spinal and autonomic nerve fibers. Each peripheral spinal nerve is the net result of union of ventral and dorsal rami of each spinal segment[(1)(2)(3)].

Each peripheral nerve consists of a bundle of nerve fibers. Each nerve fiber is wrapped by multiple layers of myelin sheath, which could reach to hundred layers. The myelin sheath formed by Schwann cells. The myelin sheath surrounded the plasma membrane of the axon which called axolemma[(2)]. The Schwann cells are responsible for myelination of the peripheral nerve fibers while oligodendrocytes take this responsibility in the central nervous system[(4)]. The main chemical constituents of myelin sheath is lipoprotein in which lipid represent 80% of its content. This lipid content is responsible for the white discoloration of nerve fiber, centrally and peripherally[(5)]. The myelin sheath does not wrap the nerve fiber continuously. Since there is a gap where myelin sheath is absent. This gap called node of ranvier. The area between two nodes called internode. Each Schwann cells are responsible for myelination of each internode area[(2)]. Each nerve trunk is surrounded by dense connective tissue called epineurium. Each nerve trunk consist of multiple fascicls, each one of them was surrounded by another connective tissue called perineurium. Each nerve fiber within thefascicls is surrounded by endoneurium[(2)]. During exposure of peripheral nerve to any type of damage, the process called peripheral neuropathy[(6)]. The diseased area leads to demyelination of nerve fibers with damage of Schwann cell and antegrade degeneration of nerve fibers[(7)]. Many treatment hand been applied to treat peripheral neuropathy. Among these medications is vitamins B[(8)(9)]. However the exact mechanism of therapeutic role of vitamins B is still questionable.

2. Aim of Study

Study the effect of each type of vitamins B (B1,B6, B12) on the repair of damaged nerve through its effect on myelin sheath and Schwann cells.

3. Materials and Methods

Forty Swiss albino rats of both sexes weighing(160 -200) had been involved in this study. All the forty animals had been exposed to crush injury of the sciatic nerve of their left hind limb. This injury had been done through a longitudinal incision in the back of the left thigh, then sciatic nerve after exploration had been crush using Kocherhemostatic forceps size 8mm for 25 second. The animals included had been divided into 4 groups as summarized in table 1:-

Table 1

Grouping of animals	Controlexperimental group	Experimental groups		
	A	B	C	D
Number of animals	10	10	10	10
Animals medication	Intermuscu-al injection of 1ml of normal saline	Intramuscular injection of 180 mg / kg body weight of vitamin B1[(10)] (ZhongshanCo.,LTD).	intramuscular injection of 180 mg / kg bodyweight of vitamin B6[(10)] (ZhongshanCo.,LTD).	intramuscular injection of 1 mg / kg bodyweight of vitamin B12[(11)] (Bushu pharmaceuticals Ltd. For Eisai Co., Ltd).
Duration of medication	45 days	45 days	45 days	45 days

Five animals from each group had been sacrificed after 30 day of experiment, while the other five ones had been sacrificed after 45 day of experiment. Apiece of 5mm of crushed area of sciatic nerve had been excised and divided into 2 pieces. The 1st piece of nerve tissue had been prepared for eosin and haematoxyllinstaning to study the histological changes occurred in the crush sciatic nerve[12].The 2nd piece of nerve tissue treated for immune-histochemical study using S 100 (DAKO polyclonal rabbit anti-100 code No.Z0311) for identification of Schwann cells[13]. In this procedure the tissue section had been labeled by using S 100 then polyclonal rabbit Anti- 100 had been used as an Antibody against S 100 Labelled Schwann cell (which act as an antigen).

Stastical analysis:-
In this study SPSS statistical analysis had been used.
P-value< 0.05 considered statistically as slightly significant
P-value< 0.01 considered statistically as moderatly significant
P-value< 0.001 considered statistically as strongly significant

4. Result

Generally, all the animals included in this study had been appeared in acceptable general condition. No infection had been reported, a part of limping, weakness of left lower limb with wasting of muscle bulk of the thigh and leg of the same limb. This observation accompanied by dragging of the dorsum of the left foot. However this had been improved with progress of the experiment, obviously in group D which treated with vitamin B12.

Histologically, the tissue sections revealed distribution of continuity of nerve fibers, degeneration of myelin sheath and disintegration of Schwann cells. Besides the area invaded with large number of macrophages with vacuole formation.

This histological picture could be demonstrated early following crush injury to the sciatic nerve (in tissue section of animal sacrificed after 30 day of experiment).

With time, tissue sections of animal sacrificed at 45 day of experiment would illustrated regeneration of the nerve tissue which could be detected histologically including Schwann cells proliferation and remyelination of newly regenerated nerve fibers , macrophage reduce in number, yet could be detected within tissue field . The nerve fiber appeared to retain its normal cytoskeleton pattern. This change had been observed frankly in tissue section of group D, followed by tissue section in group B (which is treated by vitamin B1) and the least observation had been illustrated in group C and then group A.**Fig. 2**

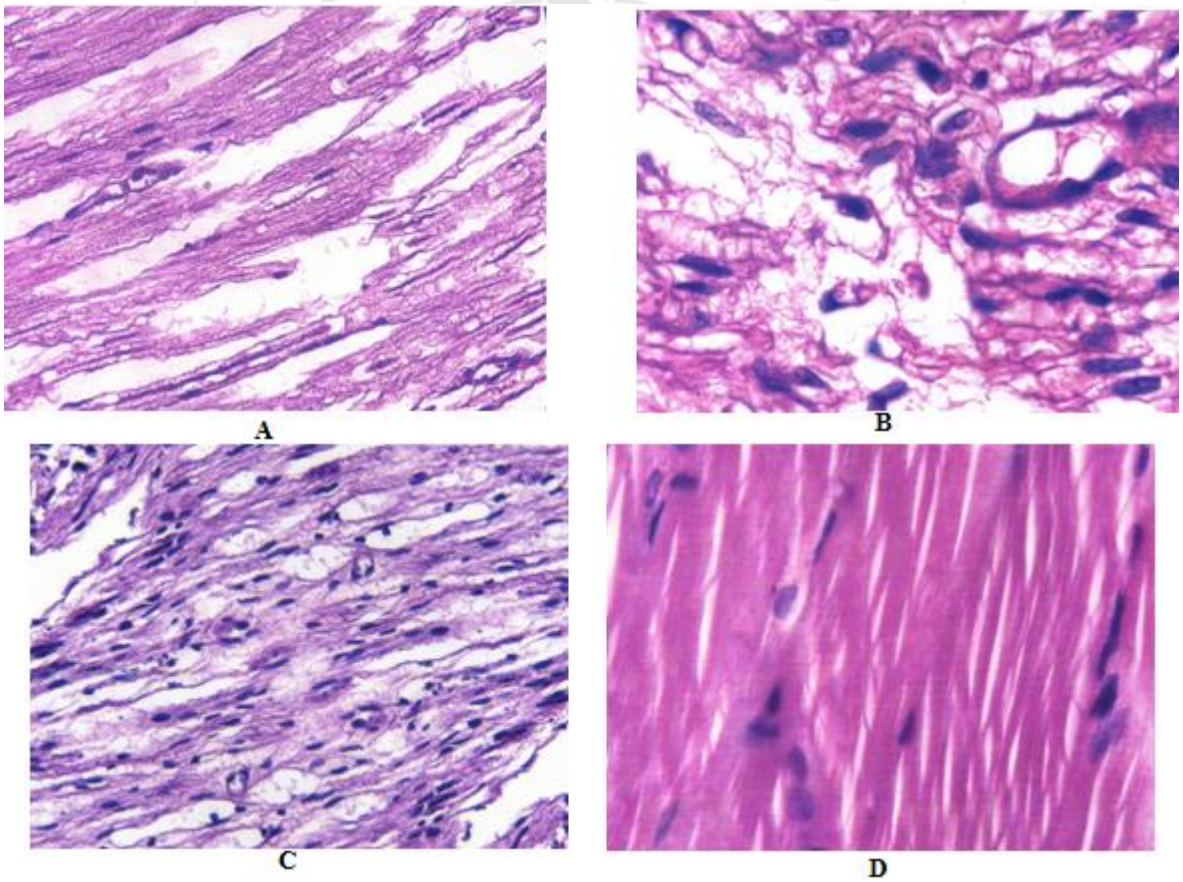


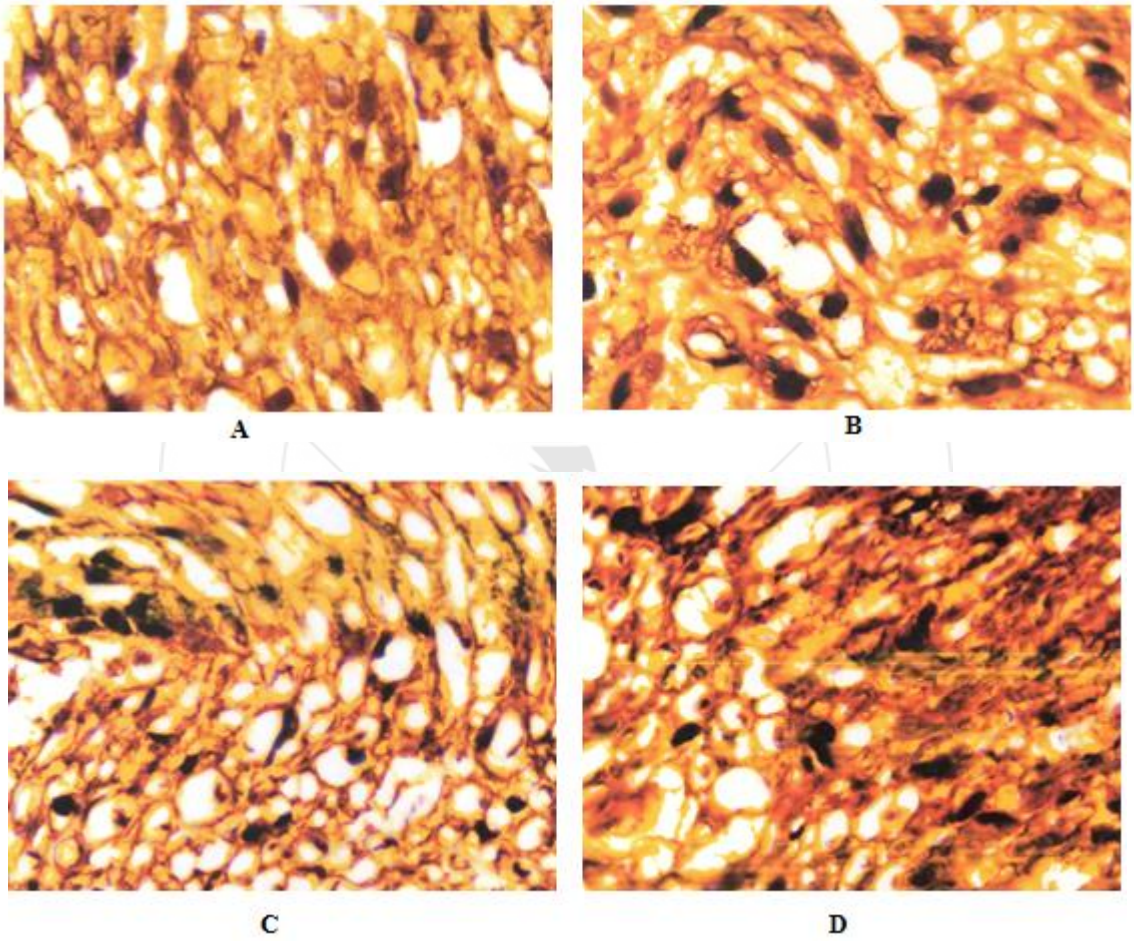
Figure 2: Nerve tissue section stained with Eosin and Haematoxilin after 45 days of experiment.
A- Normal nerve tissue section
B- Crushed nerve fiber treated with vitamin Bl
C- Crushed nerve fiber treated with vitamin B6
D- Crushed nerve fiber treated with vitamin B12

Tissue section of group B revealed little disturbance of nerve fiber with a lot of regenerated nerve fiber with many Schwann cells with little macrophage.

Tissue section treated with vitamin B6 elicited abundant of connective tissue, disintegrated nerve fibers, many macrophage and damaged Schwann cells.
Vitamin B12 treated animals illustrated almost normal cytoskeleton nerve tissue with almost normal nerve fiber arrangement, with almost absence of macrophage.

Regarding the group of tissue sections that process immunohistochemical using S 100.It demonstrated brownish – yellow discoloration of the cytoplasm with black discoloration of the Schwann cell nucleus.

Group A tissue section, whose animals exposed to crush injury without treatment, illustreated faint discoloration of cytoplasm, with many vacuoles and with few regenerated Schwann cells. While animals of group B, which was treated with vitamin B1, revealed moderate reaction when treated with S 100 ($P< 0.01$). B6 treated animals, their tissue sections elicited weak reaction ($P< 0.05$) and slightly better than that of group A reaction. While group D animals tissue section, which received vitamin B12 , it demonstrated strong reaction ($P< 0.001$) in immunohistochemical study. Their tissue sections revealed intense brownish discoloration with diffuse darkly colored Schwann cells nucleus.**Fig. 3**



Nerve fiber section stained with immunohistochemical (S 100) technique.

A- Represent control experimental tissue section showed light brownish-yellowish discoloration of cytoplasm and damaged Schwann cells.
B- Bl treated rat nerve section revealed abundant of nerve distributed fibers with dark discoloration of cytoplasm and many distributed Schwann cells.
C- Rat received vitamin B6 elicited many vacuoles with distributed nerve fibers ,faint discoloration and many dead nucleus.
D- tissue section demonstrated dark brownish-yellowish diffuse discoloration of cytoplasm with diffuse Schwann cells nuclei.

Regarding the group of tissue sections that process immunohistochemical using S 100. It demonstrated brownish – yellow discoloration of the cytoplasm with black discoloration of the Schwann cell nucleus.

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Their tissue sections revealed intense brownish discoloration with diffuse darkly colored Schwann cell nucleus.

5. Discussion

Injury to the nerve fiber lead to degeneration of myelin sheath and disintegration of Schwann cells, then if the trauma is sever,the nerve fibers will be degenerated. Schwann cells are responsible for myelin sheath formation[14][15]which has an essential role in nerve conduction along nerve fibers, besides it acts as insulator for each nerve fiber. Therefore neurologists consider Schwann cells as a road stone in nerve fiber function and have acritical role in the process of myelination[16]. Therefore following crush injury to the sciatic nerve, the 1st sign of regeneration is proliferation of Schwann cells with formation of myelin sheath[17]. However the degree of regeneration varies according to the type of medication that have been received by experimental animals.

In the current study, the tissue sections of animals received vitamin B12 elicited rapid acceptable regeneration and myelination of the nerve fibers. These finding could be seen more than that seen in the rats treated with vitamin B1, which in turn, much better than these animals received vitamin B1.

Vitamin B1 and vitamin B6 play a principle role in nervous system regeneration, however their function are restricted to the increase in energy needed for nerve conduction and production of neurotransmitter[18][19]. While vitamin B12 play a vital role in the process of neuronal regeneration through myelination of nerve fiber[20]. The transmethylation converts homocysteine to methionine which revealed a powerful affinity in transmission of nervous system[21]. Methylcobalamin enhances myelination of peripheral nerve through promotion of lecithin production which in an inactive ingredient of myelin sheath lipid component which accelerates regeneration of peripheral nerve[21][22]. Besides this vital effect of vitamin B12 in myelination of nerve fiber, it is considered as an important factor in formation of neurotransmitter[20][18].

6. Conclusion

Among vitamins B, vitamin B12 appeared to be the principle vitamin in the process of regeneration of injured nerve fiber, Since its function depends mainly on Schwann cells proliferation and myelin sheath production. Also from the current study, it was concluded that Schwann cells are the principle player in nerve fiber regeneration.

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