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Effects detraining on KIF5B gene expression in sciatic nerve fiber of male Wistar rats

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ABSTRACT: Axonal transport is a vital process in nervous system that protects axons and nerve terminals through supplying proteins, lipids and mitochondria and clearing misfolded proteins to avoid toxicity. KIF5B is one of the proteins involved in fast anterograde axonal transport. As respects to prevalence of the axonal transport proteins impairment in neurodegenerative disease, the purpose of this study is investigation of effect of decreased activity and neuropathic pain on KIF1B gene expression in sciatic nerve fiber of male Wistar rats. Ten adult male wistar rats in the weight range of 220±30 gr randomly divided into two groups including healthy control, Decreased physical activity. In the end of protocol, change of KIF5B gene expression in sciatic nerve measured with Real time technique. After 6 weeks, KIF5B gene expression in sciatic nerve ligation group compared to controls increased significantly ($P<0.05$). It seems that neuropathic pain and decreased physical activity is associate with increased KIF5B gene expression in sciatic nerve fiber. According to the physiologic functions of KIF5B in neurons, this condition may cause functional disorders in the sciatic nerves.

***Keywords*:** Detraining, Axonal transport, KIF5B, Rats.

INTRODUCTION

Neuropathic pain is a chronic pain that is defined as a pain caused by damages to or dysfunction of somatosensory system and can expresses itself in following forms: allodynia, hyperalgesia and spontaneous pains [1]. Along with causing changes to nervous system, neuropathic pain can decrease physical activity levels [2, 3]. Furthermore neuropathic pain can effect on structure and function of muscles through muscular atrophy [4, 5]. Many studies have proven that neuropathic pains' models will be followed with muscle atrophy [5-8]. However, exact cellular mechanisms which cause changes after nervous damages are still unknown [8].

Among the most prevalent complication of diabetes, neuropathy develops as an irreversible complication in more than half of with diabetes type 1 or 2 [9]. Diabetes is associated with slowing of motor nerve conduction velocity and reduced muscle contractile properties [10-11]. In contrast, on the sensory side, there is not only slowing of sensory nerve conduction velocity but also atrophy of primary and axons associated with down-regulation of structural protein synthesis and loss of terminal epidermal axons [12-13].

Several studies have demonstrated that axonal transport deficits in many neurodegenerative diseases might be due to alterations of molecular motor proteins that carry cargoes, structural and regulatory microtubules protein that serve as rail roads, adaptors and scaffold proteins that regulate cargoes attachment to motor proteins, and metabolic modification that disrupt energy supply of motor proteins[14-20]. Among different regulators of axonal motor proteins, SYD as a scaffold protein can interacts with Kinesin and mediates the axonal transport of at least three classes of vesicles [19-20]. It was shown that SYD interacts directly with the tail domain of Kinesin heavy chain and activates it for microtubule-based transport [21]. Among the 45 Kinesin motor proteins that are involved

in axonal and intracellular transport, in neuronal cells KIF5B transport synaptic vesicle precursors, membrane organelles, and mitochondria [22-23]. On the other hand, impairment of anterograde axonal transport of some neurotrophic factors and synaptic proteins is demonstrated in neuronal cells of diabetic rats [24-25]. Also, indirect evidence shows that axonal transport of mitochondria may be decreased in diabetic neuropathy[26]. Previous studies have shown that treadmill exercise training can improve peripheral nervous tissue regeneration in non-diabetic rats and mice after nerve injury and in diabetic rats, improves neuropathic pain and increases axonal regeneration after sciatic nerve transection[27-32].Exercise training is an interesting model with which increase activation of sensory and motor neurons, axonal transport of proteins, and synaptic remodeling[33-35].We have previously demonstrated that the amount of CGRP anterograde transported along axons by fast transport is increased in sciatic Moto neurons of exercise-trained rats[36].These studies have proved that exercise training increases the quantity of axonal proteins and axonal transport, but the effects of exercise training on motor proteins that transport these neurotrophic factors are not elucidated yet. Moreover, to our knowledge, no studies have been performed to analyze the effect of detraining on KIF5B motor proteins expression in sciatic nerve fiber. In the present study, we investigated KIF5B changes in sciatic nerve following the detraining.

MATERIALS AND METHODS

Animals

In present study 10 mature Wistar male rats with 10 weeks age and weight range of 250 g were provided by Animal Maintenance Unit of Razi Research Center (Razi institute Animal Center, Karaj, Iran) and conveyed to Animal Laboratory of Tarbiat Modares University. All rats were kept under a controlled environment condition with mean temperature of 22±3 C degrees, dark-light cycle of 12:12 hours, relative humidity of 40 % and free access to food and water ad libitum. The experimental protocols to perform this study were approved by the Ethics Committee on the use of animals of Tarbiat Modares University, Tehran, Iran. All efforts were made to minimize discomfort of the animals and reduce the number of experimental animals. All procedures conformed to the ethical guidelines for the care and use of laboratory animals, published by the International Association for the Study of Pain and the National Institutes of Health. After two weeks of acclimatization of animal with new environment, experimental protocols were initiated and the rats were randomly (simple randomization) allocated in three groups (5 rats in each group): (1) detraining (n=5); (2) sham surgery (Sham: n=5). Calculated sample size by the following formula showed 3 animals in each group:

N = [(Z + Z) 2Sd2] d2: Where Zα = 1.96, Zβ = 0.84, SD = 0.18 and d = 0.4. The expected power was considered at 80%.

Induction of neuropathic pain

Animals were anesthetized by pentobarbital sodium (60 mg/Kg, intraperitoneal). Then the L_5 spinal nerve was tightly ligated according to the method of Kim and Chung (1992) [37]. Briefly the left Para spinal muscles were separated at the L_5-S_2 levels and the left transverse process of the L_6 vertebra was removed. The left L_5 spinal nerve was identified and gently separated from adjacent L_4 spinal nerve. The L_5 spinal nerve was tightly ligated using silk threads (6-0) and was transected just distal to ligature to ensure that all fibers were interrupted. Then the wound closed with 3-0 silk threads. Great care was taken to avoid any damages to L_4 nerve. In a control sham group, the surgical procedure was identical to that described above, except for the left L_5 spinal nerve that was not ligated and transected. Only animals showing no signs of motor deficiencies were considered to be used for further experimentations. Only animals were chosen to continue the experiment with that had shown neuropathic pains in their behavioral tests. Then the rats were divided in 2 groups with 5 members in each: sham and detraining groups. After 6 weeks, rats anesthetized to take samples after injecting intraperitoneal ketamine (90 mL in Kg) and xialyzine (10 mL in Kg) and the muscle tissue samples separated from the left soleus muscle and were situated in -80 nitrogen for future analysis.

RNA extraction and cDNA synthesis

RNA extraction was done by QlAzol® Lysis Reagent (Germany, Qiagen) and chloroform (Germany, Qiagen) and in accordance to its manufacturer's instructions. So about 50 Mg of the muscle tissue homogenized separately in 1 to 10 portions in QlAzol® Lysis Reagent for total RNA extraction and for removing protein components. The final product was centrifuged at 12000×g for 10 minutes at 4 degrees C. Then mixed with chloroform in 1 to 5 portions and shaken severely for 15 seconds. Then the supernatant was at 12000×g for 10 minutes at 4 degrees C and its mineral part and its water removed. Finally, its RNA contained portion removed and mixed with isopropanol in 1 to 5 portions. It left for 10 minutes in room temperature and then centrifuged in 4 C degrees for 10 minutes with

12000 g revolution. RNA contained Pellet was washed and resolved in 20 microlitre RNsa-free water. RNA concentration was measured by UV spectrophotometry method (Eppendorff, Germany), and 260 to 280 portions in 1.8 -2 were determined as the desired purification. cDNA synthesis was done by using Quanti Tect Reverse Transcription Kit (Qiagen, Germany) in accordance to manufacturer's manual.

Real-Time PCR

Real-Time PCR quantity method was used by Premix SYBR Green II (Qiagen, Germany) for measuring Kif5B mRNA expression levels (Applied Biosystems Step One, America). Reaction mixture was done in final volume in 20 microliters (includes 1 microliter of cDNA, 1 microliter of forward primer, 1 microliter of reverse primer, 7 microliters of DEPC water and 10 microliters of Syber Green) and each reaction in a duplicate form. Designing primers was done according to Kif5B and GAPDH genes in gene bank of NCBI and by German company, Qiagen. Usable primer sequences have been reported: The forward and reverse primers for KIF5B gene were 5´-GATGTAAAGCAACCGGAGGGG and 5´-TGTTGGGAGATACGAAGCTGG. The forward and reverse primers for GAPDH (reference gene) were 5´-GACATGCCGCCTGGAGAAAC and 5´-AGCCCAGGATGCCCTTTAGT. Thermal program used in Real Time-PCR included: 95 degrees C for 10 minutes, 95 degrees C for 15 seconds, 60 degrees C for 1 minute (40 cycle repetitions). Melt curve and standard curve were drawn and considered for evaluating data authenticity and optimization experiment conditions respectively and Kif5B expression data were normalized using GAPDH (reference gene). Fold change of genes was measured by formula $R=2^{-ct\Delta\Delta}$ [38].

Statistical analysis

All statistical analyses were done by using SPSS software (version 19, SPSS Inc., Chicago, IL, USA). Normal assumption was examined using one-sample Kolmogorov-Smirnov test. T-tests were used to comparing groups in under study variables. Significant level was determined at α=0.05.

RESULTS AND DISCUSSION

To elucidate the possible regulation of Kif5B at mRNA level in rat models with neuropathic pain, we examined Kif5B at mRNA level in soleus muscle after 6 weeks of tight ligation of L5 spinal nerves. Indicated in Figure **1** Kif5B levels were upragulated.

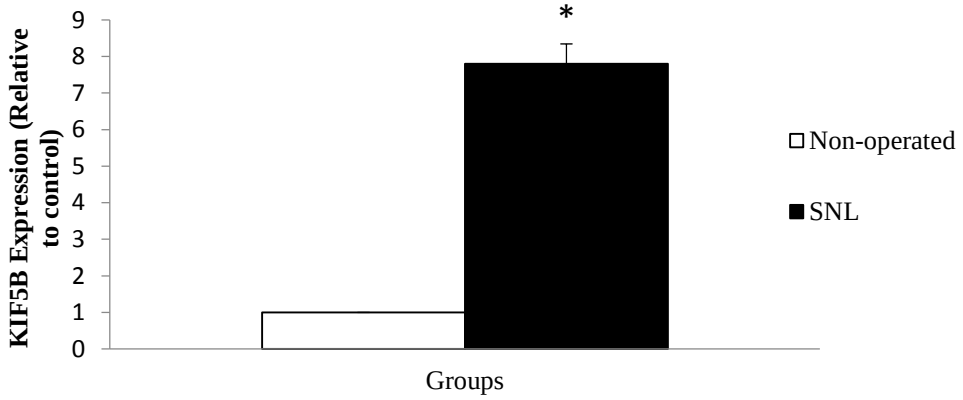

Figure 1. Real-Time amplification of Kif5B mRNA in Sciatic nerve. Data are shown as mean * which indicates significant differences with another groups (p< 0.05).

Discussion and conclusion

The results of present study are the first to demonstrate that SNL up-regulates KIF5B mRNA at sciatic nerves of rats. Moreover, physical activity modulated both KIF5B in sciatic nerves of active rats. These results indicated that sciatic nerves' KIF5B levels in SNL state could be changed and probably exercise training can modify it.

In present study, it was shown that decreased activity with tight ligation of the L5 spinal nerve cause increase in Kif1B mRNA levels. Generally increase in Kif1B probably is related to protein synthesis, and structural disorders and function of sciatic nerve. So it is likely that increased activity in form of strength and endurance training can contradict unnatural expression of these two genes in this kind of neuropathic pain. However it needs more study to state it as a certain fact.

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