The effect of endurance training on dynein motor protein expression in Wistar male rats sciatic nerves with diabetic neuropathy

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Original Article

Abstract

Introduction: Most neurodegenerative diseases are associated with the disruption of axonal transport and this might also be related to diabetes-associated disorders affecting the nervous system. Cytoplasmic dynein is a very important motor driving the movement of a wide range of cargoes toward the minus ends of microtubules. The effects of endurance training on dynein motor protein expression in Wistar male rats sciatic nerves with diabetic neuropathy were investigated.

Methods: Twenty one male Wistar rats were divided into three groups: diabetic control (DC: n=7), diabetic trained (DT: n=7) and Healthy control (HC: n=7). Two weeks post STZ injection (45 mg/kg, i.p.), rats were subjected to treadmill exercise for 5 days a week for over 6 weeks. Levels of mRNA and protein content were determined by Real time-PCR and ELISA.

Results: The blood glucose levels and dynein mRNA in both sensory and motor parts and dynein content in sciatic nerves were increased by diabetes (P≤0.05). Furthermore, exercise training modulated the dynein mRNA and dynein content to normal levels in diabetic trained rats (P≤0.05). Moreover, exercise training reduced blood glucose levels in the DT rats (P≤0.05).

Conclusion: The results of the present study confirm that dynein expresses up-regulation in STZ-induced diabetic rats. Increase in the amount of dynein expression can be a compensatory response in the lower trophic support and burden imposed on autophagy system. These alterations suggest that retrograde axonal transport may be impaired in diabetic rats. In addition, endurance training as a non-pharmacotherapy strategy can modulate these alterations.

Key words: Diabetic Neuropathy, Motor Proteins, Dynein Motor Proteins, Gene Expression

Introduction:

Among the most prevalent complication of diabetes, neuropathy as an irreversible complication develops in more than half of patients who have type 1 or 2 diabetes (1). Apart from damaging peripheral and central neurons, diabetes also impairs neuronal capacity for regeneration. Prior to significant motor neurons impairment, diabetes damages sensory neurons. However, it finally develops loss of neuromuscular junction (2).

Sensory neuronal loss is associated with ulceration and amputation. Later motor abnormality leads to muscle atrophy and weakness of distal leg and foot muscles (3,4). Slowing of motor nerve conduction velocity and decreased muscle contractile properties are associated with diabetes. On the other hand, on the sensory side, slowing of sensory nerve conduction velocity is not only possible (5), but also, atrophy of perikarya and axons are associated with down regulation of the synthesis of structural protein and loss of terminal epidermal axons (2,6). Distal axon targeting in diabetic neuropathy may be due to axonal transport of structural and plasticity related proteins that fall below the threshold needed to maintain the viability of distal branches (2).

Cytoplasmic dynein is a microtubule minus end-directed motor, consistent with a role in moving cargo from the cell periphery toward the cell center. As discussed below, this suggests a key role for dynein in retrograde signaling, like neurotrophic factor-induced signaling pathways, as well as in bulk transport from synapse back to cell body along the axon (7,8). Direct or indirect evidence of dynein in neurodegenerative diseases like amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease, Alzheimer’s disease, Parkinson’s disease, diabetic and Huntington’s disease have been shown in several studies (7). Features of neurodegenerative diseases are also demonstrated by dynein heavy chain mutant mice. In addition, defects of dynein-dependent processes like autophagy or clearance of aggregation-prone proteins are prevalent in most of these diseases. Lines of evidence have also demonstrated that dynein is associated with neurodevelopmental diseases (7,9). On the contrary, the impairment of retrograde axonal transport of some neurotropic factors and synaptic proteins are shown in neuronal cells of diabetic rats (10). In addition, indirect evidences revealed that axonal transport of mitochondria may be reduced in diabetic neuropathy (11).

Past studies have demonstrated that treadmill exercise training can enhance peripheral nervous tissue regeneration in non-diabetic rats and mice after nerve injury (12-14) and in diabetic rats, enhance neuropathic pain and increase axonal regeneration after sciatic nerve transection (15,16).

Exercise training is an interesting model which increase activation of sensory and motor neurons, axonal transport of proteins, and synaptic remodeling. It has been previously revealed that the amount of CGRP anterogrately transported along axons by fast transport is increased in sciatic motoneurons of exercise-trained rats (15,17). These studies have shown that exercise training increase the quantity of axonal proteins and axonal transport, however, the effects of exercise training on motor proteins that transport these neurotrophic factors are not yet elucidated. In addition, to the best of our knowledge, no studies have been carried out to analyze the effect of exercise training on dynein motor proteins expression in diabetic neuropathy. In the present study, the effects of endurance training on dynein motor protein expression in Wistar male rats sciatic nerves with diabetic neuropathy were investigated.

Methods:

Animals characteristics

Experiments were performed on 300 to 320-g male Wistar rats (Razi institute Animal Center, Karaj, Iran). The rats were housed at a constant room temperature of 22±2°C under a 12-h light/dark cycle (lights on at 6:00 AM), with access to food and water ad libitum in the Animal Center of Tarbiat Modares University. 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 discomort 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 National Institutes of Health.
Groups and Design
Twenty one male Wistar rats were divided into three groups: diabetic control (DC: n=7), diabetic trained (DT: n=7) and Healthy control (HC: n=7). Two weeks after inducing diabetes, 6 weeks of endurance training protocol was performed. For familiarization; Rats in all groups were adapted to the treadmill by running for 5 days. This protocol was designed as once a day for 10 min/session at a speed of 10 m/min at a slope of 0 degree.

Induction of diabetes
Following an overnight fast, diabetes was induced in the hyperglycemic groups by a single intraperitoneal (i.p) injection of STZ (45mg/kg; Sigma, St. Louis, MO). STZ solution was prepared freshly by dissolving it in 0.5 mol/L citrate buffer (pH 4.0). Control animals received an equivalent volume of citrate buffered solution (15,18). As the dose of STZ used and the weight of the rats before STZ treatment are two major factors in causing diabetic complications but not profoundly ill in diabetic rats, in this study a lower dose of STZ and appropriate with rat’s weight was used to reduce the severity of illness and the side effects to an acceptable level. Diabetic condition was assessed by serial measurement of non-fasting tail vein blood glucose level using an Accu-Chek Compact Plus blood glucose meter (Roche Diagnostics K.K., Tokyo, Japan) up to 8 weeks after STZ injection, and diabetic rats with constant hyperglycemia (blood glucose levels of more than 350mg/dL) were included in the study. The body weight and general health was monitored and all animals showed suitable behavior during the entire period of the study (15).

Exercise training protocol
Training groups were performed moderate-intensity treadmill exercise 5 days a week. The speed were gradually increased from 10 m/min for 10 min (grade 0%) in the 1st week to 10 m/min for 20 min (grade 0%) in the 2nd week, 14–15 m/min for 20 min (grade 0%) in the 3rd week, 14–15 m/min for 30 min (grade 0%) in the 4th week, and 17–18 m/min for 30 min (grade 0%) from the 5th week. Protocol included 2 to 3 minutes of warm-up and 3 minutes of cool-down (15, 20).

Tissue Preparation
24 h after the last training session, animals were anaesthetized with a mixture of Ketamine (75mg/kg-1) and Xylazine (5 mg/kg-1) which was administered intraperitoneally. The lumbar enlargement (L4-L6) was isolated and sensory and motor (or frontal and dorsal) parts were separated using the central canal as a reference (15,21). And tissues were stored at -70°C until analyzed.

Real-Time PCR
Motor and sensory neuron tissues were transferred to 1 mL lysis reagent (Qiazol; Qiagen) and were homogenized (Tissue-Tearor; Biospec, Bartlesville, OK). RNA extraction was performed identically for both tissues using a purification kit (RNeasy Mini Kit; Qiagen) in accordance with the manufacturer’s instructions. Motor and sensory parts mRNA was pooled by successively adding ethanol-precipitated RNA (RNeasy; Qiagen). Concentrations of RNA were determined by UV spectrophotometry (Eppendorff, Germany). Total cDNA synthesis was performed using ABI PRISM cDNA archive kit in 0.2 ml microfuge tubes. The reaction mixture of 20 μl contained 0.2 μg total RNA, 10 × RT buffer, 25 × dNTP mixture, 10 × random primers, MultiScribe RT (50 U/μl) and RNase free water. The cDNA synthesis reactions were carried out for 20 minutes at 42°C, followed by denaturation for 5 minutes at 95°C and 5 minutes at 4°C.

Real-time PCR assays were performed in 48-well plates in ABI 7300 real-time PCR instrument (Applied Biosystems). The primers and probes were purchased from Applied Biosystems, Foster City, California, USA. The forward and reverse primers for dynein gene were 5’- CGT GGC TCT TTG AAG GGG AC -3’ and 5’- GTT GAA AAG GGC TGG TGA CG -3’. The forward and reverse primers for GAPDH (reference gene) were 5’- GACATGCGGCCCTGGAGAAC and 5’- AGCCAGGATGCCCTTATTAG. The TaqMan reaction mixture of 20 μl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer, and TaqMan probe for target gene and endogenous control (GAPDH) and 12.5 μl of Taqman 2 × Universal PCR Master Mix (Applied Biosystems) and the volume was made up with RNase free water. The following thermal
cycling profile was used (40 cycles): 50°C for 2 min, 95°C for 10 min, 95°C for 15 sec and 60°C for 1 min. The quality of the real-time PCR reactions was confirmed by melting curve analyses. Efficiency was determined for each gene using a standard curve (logarithmic dilution series of cDNA from the tests). For each sample, the reference gene (GAPDH) and target gene were amplified in the same run. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalize with the control (ΔΔCT = ΔCT – CTControl). The fold change in expression was then obtained as 2ΔΔCT (15, 21).

**Dynein Assay**

Dynein tissue homogenized (1: 10 in 10 MmPBS, pH 7.4 at 4°C) and centrifuged (20000 rpm/45 min) sciatic samples were determined with Rat cytoplasmic dynein heavy chain 1 (DYN1H1) ELISA kit (CUSABIO, CusAb, US). CSB-EL007292RA, Detect Range: 31.25 pg/ml-2000 pg/ml, Sensitivity: 7.8pg/ml according to the manufacturer’s instructions.

**Statistical Analysis**

Data were analyzed by SPSS16.0 for Windows (SPSS Inc., Chicago, IL). Data are presented as mean±SEM. Descriptive 2-way analysis of variance (ANOVA) was used in different groups. If analysis was significant, the differences between groups were estimated using LSD’s post hoc test. Significance was defined as P<0.05.

**Results:**

**Body weight and Blood glucose levels**

All rats in training groups completed the 6-week endurance training program. Compared with HT and HC groups, the DT and DC groups developed significantly reduced body weight (P=0.0001 and P=0.001 respectively). Also, the weight of DT group than in DC group was significantly lower (P=0.04) (Table 1). The change in blood glucose levels is shown in Table 1.

Diabetic rats developed hyperglycemia within 2 days after STZ injection. Before training, blood glucose concentration was significantly higher in diabetic groups compared with normal groups (P=0.0001), and after 6 weeks of endurance training was still statistically significant (P=0.0001). Also, after training period, blood glucose concentration was significantly lower in DT compared with DC (P=0.0001) (Table 1).

**Dynein mRNA levels**

Real Time-PCR analysis showed that compared with normal control, Dynein mRNA levels were elevated 4.88-fold in motor parts (P=0.001) and 3.43-fold in sensory parts of DC rats (P=0.001) and endurance training could significantly modulate them in sensory and motor parts of DT rats (P=0.001 and P=0.001 respectively) (Figure 2,3).
Figure 1. Components of the microtubule-based axonal transport system (a: Kinesin and dynein complexes, b: Cargoes including mitochondria and vesicles) (26)

Figure 2. Representative graph showing Real-Time amplification of dynein mRNA in sensory parts. (* and †)=indicate significant differences with HC and DT respectively (P ≤ 0.5)

Figure 3. Representative graph showing Real-Time amplification of dynein mRNA in motor parts. (* and †)=indicate significant differences with HC and DT respectively (P ≤ 0.5).

Dynein content in sciatic nerve

Diabetes leads to significant increase in Dynein content of sciatic nerve in DC rats compared to HC rats (P=0.001). However, endurance training significantly were decreased to normality but no significant difference between dynein content of sciatic nerve in DT and HC rats were observed (P=0.55) (Table 1).

Conclusion:

This study tested the hypothesis that diabetes and exercise can have effect on the axonal motor protein involved in neuronal transport of synaptic proteins, mRNA and mitochondria in sensory and motor neurons of spinal cord of STZ-induced diabetic rats.

The results of this study are the first to show that diabetes up-regulates dynein mRNA at lumbar sensory and motor neurons and protein levels at sciatic nerves of STZ-induced diabetic rats. The reduced retrograde transport could contribute to the motor neuron death by reducing the removal of damaged mitochondria from the synapse or by decreasing the level of survival signals induced by neurotrophic factors secreted by the muscle. Dynein is major motor protein that is responsible for retrograde transport, the alterations in the retrogradely transported cargos may be an alternative mechanism contributing to motor neuron death (9,22). Apart from its role in retrograde axonal transport, the dynein–dynactin complex also have other numerous functions, such as participation in mitosis, endoplasmic reticulum to golgi vesicular trafficking, neuronal migration, neurite outgrowth, formation of synapse, aggresomes formation, and degradation of protein by autophagy (9,22,23).

The slow transport of neurofilaments has been suggested to occur in the ‘tug-of-war’ manner while cargos like peroxisomes and vesicles have been suggested to be transported in a coordinated manner (7,24). The coordination of the motor activities...
could be regulated by direct interaction between motor proteins, adaptor proteins linking different motors, or by other regulatory pathways. Recent studies have given evidence regarding all the proposed mechanisms. A direct interaction between kinesin-1 and dynein has been observed (7,15).

Nevertheless, although motor proteins with up-regulation of retrograde axonal transport is possible in diabetes, kinetics may be impaired (25).

Decreased in the content of KIF1A after 8 weeks of diabetes has been reported. With regard to dynein immunoreactivity, there was an increase in the ganglion cell. It has been suggested that axonal transport may be impaired in diabetes, which might contribute to early signs of neural dysfunction in the retina of diabetic patients and animal models (10).

The expression of NT-3 mRNA in the dorsal root and soleus muscle is increased in diabetes. It is suggested that diabetes may elevate NT-3 mRNA production while neurotrophic support for NT-3 may be impaired (16).

Maintenance and function of neurons are dependent on the intracellular transport of organelles along the axons. Basically, having a good synaptic function and mitochondria for local energy requirements in neurons are attributed to molecular motor proteins that anterogradely transport cargoes (26). Significant defects in synaptic function and “dying-back” degeneration of axons are characteristics of neurodegenerative diseases (27,28). Accordingly, alterations in axonal transport have been documented in Alzheimer’s, Parkinson’s, Huntington’s diseases, amyotrophic lateral sclerosis (7,10). These diseases are associated with mutations/dysfunctions in molecular motor proteins or alterations in the activity of specific protein kinases that have been implicated in the regulation of these motor proteins’ function (26,27).

One of the most important roles of these motor proteins is transporting of synaptic vesicles. Our results demonstrate that diabetes might alter molecular motor which is involved in synaptic function and exercise can modify this condition. Confirming these data, synaptic degradation of nerves was shown in STZ-induced diabetic rats (29,30). In addition, the activity-dependent regulation of gene expression occurs even in the adult brain and it is believed to play an important role in synaptic plasticity. Increased afferent inputs of a neuron by exercise training or electrical stimuli bring long-term potentiation (LTP) as a form of long-lasting synaptic plasticity adaptation in both CNS and PNS. It is shown that synthesis of new mRNA and proteins are important for the induction of LTP (31,32). Furthermore, several studies have shown that the induction of synaptic plasticity are dependent on the transcription of many genes (33).

Mitochondria are another cargo transported mainly by dynein motor protein in the neuronal cells. Mitochondria complex mobility patterns within neurons are characterized by frequent changes in direction (29). Defects in mitochondrial transport are implicated in pathogenesis of several neurodegenerative diseases; such as Huntington’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, and Charcot–Marie–Tooth disease. Moreover, mitochondrial dysfunction has been proposed as a major mediator of neurodegeneration in diabetes and indirect evidences revealed that axonal transport of mitochrondria may be reduced in diabetic neuropathy (11,22). Our results suggest that diabetic state changes sciatic levels of dynein motor protein which is involved in axonal transport of mitochondria.

Interestingly, our results reveal that endurance exercise training can up-regulate expression and protein content of dynein motor proteins in sensory and motor neurons and sciatic nerves of non-diabetic rats, respectively. We have previously revealed that the amount of anterograde axonal transport of CGRP (17), which is a dynein cargo, is increased in sciatic motoneurons of exercise-trained rats. Although direct evidence of the mechanism of hyperglycemia to alter dynein expression in this study was not provided, accumulated evidence demonstrates that hyperglycemia seems to be an essential factor for neurodegeneration in diabetes, which has been associated with decreased axonal transport rates in STZ-induced diabetic mice (20,34). However, the present study showed that endurance training exercise significantly reduce blood glucose levels in STZ-induced diabetic trained rats.

Axonal transport defects in diabetic mice were attributed to hyperglycemia-induced oxidative stress
damages (20). Chronic hyperglycemia induces ischemic, hypoxic, oxidative, and apoptotic stress leading to widespread damage to proteins, cells, and tissues. Chaperone molecules also play a crucial role in minimizing hyperglycemic stress (35).

Physical activity can improve antioxidant system, increase mitochondrial biogenesis and reduction of inflammatory cytokines. Endurance training by improving levels of cellular energy, and decreasing of inflammatory cytokines, increase the antioxidant capacity of cells and ultimately prevent protein aggregation and decrease the burden imposed on autophagy system leading to downregulation in the expression of dynein motor protein during endurance training (13,19,32,36,37).

The results of the present study and other researches in the field of axonal transport impairments in neurodegenerative diseases may be supportive of “cargo and driver theory” (15). This theory explains that in neurodegenerative diseases including diabetic neuropathy, impaired axonal transport is attributed to two reasons. First: downregulation of neurotrophic factors and other proteins which are important for neuronal survival. Second: impairment in motor proteins that actively transport these cargoes (15,26,27,37). Another important issue related to this theory is the modifying role of exercise. Nonetheless, there are other related points which should be explained by future studies.

The results of the present study confirm that dynein expresses up-regulation in STZ-induced diabetic rats. Increase in the amount of dynein expression can be a compensatory response in the lower trophic support and burden imposed on autophagy system. These alterations suggest that retrograde axonal transport may be impaired in diabetic rats. In addition, endurance training as a non-pharmacotherapy strategy can modulate these alterations.

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نگهداری

مقدمه
اختلال در حمل و نقل آکسون با بسیاری از بیماری‌های عصبی مرتبط است؛ همچنین ممکن است نقش مهمی در اختلالات نروپاتی دیابتی داشته باشد. داینئین اولین و عمده ترین موتور حرکتی در انتقال رو به عقب اکسونی است که با انتقال اندامک‌های گلوکز، افکار و نورون‌ها نقش اساسی دارد. هدف از پژوهش حاضر، بررسی تأثیر تمرین استقامتی بر بیان پروتئین حرکتی داینئین در عصب سیاتیک موش‌های صحرایی نر ویستار دارای نروپاتی دیابت بود.

روش‌کار
31 سر موش صحرایی بالغ نر نژاد ویستار به طور تصادفی در سه گروه هفت‌تایی: دیابت کنترل، دیابت تمرین و سالم کنترل قرار گرفتند. جهت قرار گرفتن نتوانستگی در حمل و نقل آکسون با بیماری‌های عصبی مرتبط است که با انتقال اندامک‌های گلوکز، افکار و نورون‌ها نقش اساسی دارد. هدف از پژوهش حاضر، بررسی تأثیر تمرین استقامتی بر بیان پروتئین حرکتی داینئین در عصب سیاتیک موش‌های صحرایی نر ویستار دارای نروپاتی دیابت بود.

نتایج
دیابت باعث افزایش معنادار در سطح گلوکز خون، بیان mRNA پروتئین حرکتی داینئین بخش حس و حرکتی و محتوی پروتئین داینئین در عصب سیاتیک موش‌های صحرایی نر ویستار دارای نروپاتی دیابت بود.

نتیجه‌گیری
نتایج پژوهش حاضر نشان می‌دهد که داینئین یکی از پروتئین‌هایی است که در حمل و نقل آکسون با بیماری‌های عصبی مرتبط است. هدف از پژوهش تمرین استقامتی بر بیان پروتئین حرکتی داینئین در عصب سیاتیک موش‌های صحرایی نر ویستار دارای نروپاتی دیابت بود.

کلیدواژه‌ها: نروپاتی دیابت، پروتئین حرکتی داینئین، بیان mRN4.