Effects of Insulin on Fibronectin Alterations in Sciatic Nerve of Diabetic Rats-A Brief Report

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Abstract

Objective: Alteration in the basement membrane proteins maybe associated with diabetic neuropathy. Fibronectin is one of the most important components of peripheral nerves basement membrane. In this study we investigated the effects of insulin administration on prevention of alteration in fibronectin contents of sciatic nerve in diabetic rats.

Materials and Methods: Twenty-four wistar rats were divided into control, diabetic and diabetic with insulin treatment groups. Three months after diabetes induction, we measured blood glucose level, body weight and then expression of fibronectin in sciatic nerves of rats were evaluated by real time polymerase chain reaction (PCR) and immunohistochemical study.

Results: Intensity of fibronectin immunoreactivity in the perineurium and endoneurium of sciatic nerves significantly increased in diabetic without treatment group compared to control group (P-value< 0.001).

Conclusion: This finding suggested that diabetic neuropathy resulted in increased of fibronectin contents in sciatic nerves of rats.

Keywords: Diabetes, Sciatic nerve, Basement membrane proteins, Fibronectin

Introduction

Change in basement membrane thickening of peripheral nerves may be seen during diabetes (1,2). Basement membranes are specialized form of extracellular matrix (ECM). ECM provides physical support for cells and tissue. Increased thickness of perineurial cell basement membrane may alter perineurium rigidity and change in blood vessels structure that lead to changes in oxygen supply and nutrients to the endoneurium (3). Fibronectin is a prominent glycoprotein in many of extracellular matrix. It involves in many processes including cell adhesion, morphology and migration (4). The skeleton of perineurial cell basement membranes are mainly formed by collagen IV, laminin and fibronectin (5,6). Hyperglycemia may be resulted in advanced glycation end products (AGEs) formation. The further AGEs formation may be leads to changes in components of the extracellular matrix such as fibronectin (7). This study investigated the

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alterations of fibronectin contents in sciatic nerves of diabetic rats and effects of insulin administration to prevent or reverse of these alterations.

Materials and Methods

Twenty-four adult male wistar rats with 200–250g body weight were randomly divided into three groups: control, diabetic without treatment and diabetic with insulin treatment. Insulin administration group received 4 to 6 units of NPH insulin (EXIR Co. Iran) daily for 3 months. Fibronectin reaction in the sciatic nerves was graded according to staining intensity that described in the previous studies (8,9). In real time PCR study, nerve samples were collected to RNA later (Qiagen, Germany). Total RNA was isolated by the RNeasy Mini Kit (Qiagen, 74104) according to the manufacturer's instructions. The nerves were homogenized (Polytron 1200E, Switzerland) and were centrifuged. RNA extractions were performed and then the first strand cDNA were made by using a cDNA synthesis kit (Fermentas). The tubes were sequentially incubated for 15 min at 25oC followed by 60 min at 42oC and terminated reaction by heating at 70oC for 5 min. Finally cDNA samples were stored at -20oC. Real-time PCR was done by the Stratagene Max 3000p (USA).

The cDNA was denatured at 95°C for 10 min followed by 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 45 seconds. After final cycle, the temperature was 95°C to construct a melting curve. The cDNA content in each specimen was determined by using a comparative cycle threshold (Ct) method. The results were presented as relative expression of a specific gene normalized to the GAPDH gene. The average of the relative amount of each mRNA in control group is defined as 1.0. Briefly, the primers efficiency was calculated using serial dilution of cDNA and CT values by following formula:

Efficiency = 10 (-1/slope)-1. As our data showed the primers efficiency for target gene and endogenous control (GAPDH) was in similar range and ≥ 99%. Melting cure analysis shows a single PCR product and specificity of primers for GAPDH and target gene. The serial dilutions of cDNA from the high quality sample were used to construct a relative standard curve for the target genes and then fold change in fibronectin gene expression was calculated by comparative Ct ($2^{-\Delta\Delta Ct}$) method (10).

Ethical considerations

All experimental protocols were approved by Mashhad University of medical sciences ethics committee for animal experiments (code: 89761).

Results

Immunohistochemical study showed that significantly elevated of fibronectin reactivity in the perineurim and endoneurium of sciatic nerve in diabetic group without treatment comparison to the control group (*P*-value< 0.001), this reactivity was stronger in the perineurim compare to endoneurium. Fibronectin immunoreactivity in perineurim and endoneurium of diabetic rats with insulin treatment significantly decreased than diabetic rats without treatment (*P*-value< 0.05). (Table 1). Evaluation of real-time PCR study was showed significantly elevated of fibronectin mRNA level in diabetic group without treatment (0.5 fold) compared to control group. Insulin administration significantly overall decreased of fibronectin mRNA level

Table 1. Comparison of fibronectin immunoreactivity in the sciatic nerves of different groups

Group	Perineurium	Endoneuriun
Control	++	++
Diabetic	++++^{x}	^{x}+++
Diabetic with insulin treatment	+++$^{\#}$	++$^{\#}$

N=8 for each group; ++++: very strong expression, +++: strong expression, ++: moderate expression
P*-value<0.05: compare to control group, **# *P*-value<0.05: compare to untreated diabetic group.

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in sciatic nerve of diabetic rats (*P*-value<0.05).

Discussion

Alteration in the extracellular matrix composition is one of the most important causes of unsuccessful nerve regeneration in diabetic neuropathy (1,11-13). The skeleton of perineurial cell basement membranes are mainly formed by collagen IV, laminin and fibronectin (5). The basement membranes are special form of ECM and the disturbance of ECM components play an important role in the developing of disease in kidney and retina. Excessive accumulation of fibronectin in kidney and retina were noticed after diabetes induction (2,14,15). Our immunohistochemical results showed that hyperglycaemia could change expression of fibronectin strongly in perineurium and moderate in the endoneurium, but insulin therapy significantly decreased this over expression. This finding also showed that, fibronectin at mRNA level was significantly increased in the sciatic nerve of untreated diabetic rats. Insulin administration was significantly reduced fibronectin over expression at mRNA level. Hyperglycemia can increase expression of extracellular proteins such as Collagen IV and fibronectin (15). In experimental diabetic neuropathy oxygen free radicals increased in sciatic nerve (16,17). Hyperglycemia induced oxidative stress and AGEs formation that contributes to the pathology of diabetic neuropathy. AGEs may induce synthesis and degradation of extracellular matrix components and resulted in accumulation of collagens, fibronectins, and laminins that leading to poor axonal regeneration (7,16). Hyperglycemia and AGEs accumulation may cause alteration in the structure and function of ECM proteins in peripheral nerve (7,17,18). Recent studies have shown AGEs maybe damage function of pancreatic β-cell and decrease cell viability which leads to reduce of insulin content and secretion (19,20). Hyperglycemia and glycosylation produces cross linkage and physical alteration in properties of extracellular matrix (21,22). Connective tissue growth factor (CTGF) is up regulated in diabetes and induced by hyperglycemia and AGEs. CTGF mediates the alterations of ECM components during hyperglycemia (23). Insulin administration causes down regulated of CTGF and this decrease can effect on ECM components and inhibited up regulation of several components of ECM such as fibronectin (23).

Conclusions

Regarding the results of the present study, diabetic neuropathy resulted in increased fibronectin expression in sciatic nerve of rats. It is suggested that insulin therapy reversed fibronectin up regulation and may be useful in prevention of fibronectin alteration in peripheral nerves of diabetic rats.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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