Diabetes & Metabolic Syndrome: Clinical Research & Reviews xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Insulin-induced upregulation of lipoprotein lipase in Schwann cells during diabetic peripheral neuropathy

Kuruvanthe S. Rachana, Mallahalli S. Manu, Gopal M. Advirao*

Department of Biochemistry, Davangere University, Davangere, Karnataka, India

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Diabetic peripheral neuropathy
Insulin
Schwann cells
Lipoprotein lipase
Sciatic nerve
Myelin

ABSTRACT

Diabetic peripheral neuropathy (DPN) is one of the major complications associated with diabetes. It is characterized by the degeneration of the myelin sheath around axons, referred to as demyelination. Such demyelinations are often caused by reduced lipid component of the myelin sheath. Since, lipoprotein lipase (LPL) provides the lipid for myelin sheath by hydrolysing the triglyceride rich lipoproteins, and also helps in the uptake of lipids by the Schwann cells (SCs) for its utilization, LPL is considered as the important factor in the regeneration of myelin sheath during diabetic neuropathy. Earlier reports from our laboratory have provided the insights of insulin and its receptor in SCs during diabetic neuropathy. In order to evaluate the long term effect of insulin on lipid metabolism during diabetic neuropathy, in this study, we analyzed the expression of LPL in SCs under normal, high glucose and insulin treated conditions. A decrease in the expression of LPL was observed in SCs of high glucose condition and it was reversed upon insulin treatment. Histochemical observations of sciatic nerve of insulin treated neuropathy subjects showed the improved nerve morphology, signifying the importance of insulin in restoring the pathophysiology of diabetic neuropathy.

1. Introduction

Diabetes mellitus is one of the causes of demyelination of neurons, which often leads to neuropathy [1,2]. Increased polyol pathway and decreased function of Schwann cell (SC) mitochondria are the known causing factors for diabetic peripheral neuropathy (DPN) till date [3,4]. The glial cell of peripheral nervous system (PNS), SC is involved in maintaining the neuron health by forming myelin sheath around axon through differentiation of SC plasma membrane [2,5]. During diabetes, Schwann cells (SCs) undergo stress and could not function in regular way to replenish the lost myelin lipids, which are the major component of myelin. This induce degeneration of myelin sheath, referred to as demyelination [6]. Such alterations in the composition of myelin sheath leads to impaired function of neurons in DPN [7].

The lipid for the myelin is provided by circulating triglyceride rich lipoproteins similar to other tissues [8]. Lipoprotein lipase (LPL) is a key enzyme that helps in the hydrolysis of triglyceride rich fatty acids like circulating chylomicrons and VLDL [9]. It also helps in the uptake of lipids by cells in many tissues like heart, muscle, adipose tissue and mammary glands [10]. LPL has been detected in brain tissue, suggesting its involvement in cellular lipid synthesis in central nervous system [11]. Previous work has reported the presence of LPL enzyme in SCs and sciatic nerves, indicating its role in PNS. LPL present in the SC is known to be involved in the biosynthesis of myelin phospholipids [9,12]. It was also reported that, during diabetes, there is reduction in the activity of LPL, which may in turn reduce the uptake of lipids by glial cells and may be the factor of pathophysiology of neuropathy [8].

Insulin is known to be a neurotrophic factor, as it upregulates major myelin proteins like myelin basic protein and myelin associated glycoprotein during diabetic neuropathy [13]. It is known to enhance the activity of LPL in other tissues like adipose tissue and skeletal muscle [14,15]. As SCs play a major role in the maintenance of myelin sheath, it becomes important to know the changes that occur in SC components, which inturn affect the myelin. Short term treatment of insulin is reported to increase the activity of LPL in diabetic neuropathy in *in vivo* system. Streptozotocin induced rats have shown the corrected LPL activity upon insulin treatment for 4 days [8]. But the effect of insulin on the expression of LPL in cultured SCs (*in vitro*) are still unknown. Hence, the present study was performed in order to understand the role of insulin in enhancing the expression of LPL in SCs that are mimicking diabetic condition *in vitro,* and also to evaluate the long term effect of insulin on streptozotocin induced diabetic neuropathy rats. Results showed that insulin increased the LPL levels in *in*

* Corresponding author.
E-mail address: muttagigopal@yahoo.co.in (G.M. Advirao).

https://doi.org/10.1016/j.dsx.2018.03.017
1871-4021/

vitro systems, clearly suggesting the role of insulin in regeneration of myelin sheath in diabetic condition.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Sigma (USA) unless mentioned. DMEM and Fetal Calf Serum (FCS) were procured from Gibco (USA). Real time PCR reagents were obtained from Invitrogen (USA) and antibodies were from Millipore (India). Streptozotocin, Dulbecco's phosphate buffered saline (DPBS) and Bovine Serum Albumin (BSA) were procured from Himedia (India).

2.2. Animals and grouping

All studies were performed using Wistar rats. Rats were purchased from the Animal facility, SS Institute of Medical Sciences and Research Centre, Davangere, India. The animals were maintained in the animal facility with controlled temperature and humidity at a 12/12-h light/dark cycle with free access to food and water. Animal care and procedures for animal experiments were carried out according to the guidelines of institutional animal care and use. Pups of 3–4 day old were used for the Schwann cell culture preparation. All efforts were made to reduce the number of animals used and their suffering. Animals were categorized into three groups [Group 1, Control (C); Group 2, streptozotocin induced diabetic rats (STZ); Group 3, insulin treated diabetic rats (STZ + I)] of 12 animals each.

2.3. Diabetes induction and insulin treatment

Diabetes was induced to adult Wistar rats weighing 200–250 g by injecting 20 mg/kg body weight of streptozotocin after overnight fasting. A second dose of 40 mg/kg body weight of streptozotocin was injected after one week. Citrate buffer (0.1 M) was injected intraperitoneally to age matched rats which were used as control. After 48 h of second dose of streptozotocin, blood glucose of rats was estimated to confirm diabetes. Rats with mean plasma glucose level above 250 mg/dl were considered as diabetic. Hot plate analysis was performed as described earlier [16] to confirm DPN. Group 3 rats were daily administered with insulin (1 IU) subcutaneously. The sciatic nerves were collected from all the three groups upto 3 month at one month interval.

2.4. Isolation and culture of primary Schwann cells

Primary SCs were cultured from the sciatic nerve of 4 day old pups as previously described by Brockes et al. [17], with slight modifications [18,19]. Briefly, the sciatic nerve from postnatal day 4 was enzymatically dissociated with collagenase (0.05%) and trypsin (0.25%), and plated on poly-L-lysine coated culture dishes in DMEM with 10% FCS. Pure SCs were obtained by treating the 24 h culture with 10 μM cytosine arabinoside, a mitotic inhibitor for 12 h, which kills rapidly diving fibroblast cells. Later, cells were centrifuged and pellet was washed in calcium and magnesium free DPBS to remove the serum containing media. Then the cells were suspended in serum free 1:1 DMEM and Ham's F12 media containing gentamycin (40 mg/l), progesterone (20 nM), putrescine (100 μM), sodium selenite (30 nM), transferrin (5 mg/l) and BSA (1% w/v). Cells were seeded at a density of 30,000–40,000 on 18 mm coverslip pre coated with poly-L-lysine in a 6-well plate for immunocytochemical studies. For western blotting and real time PCR studies, cells were plated at a density of 10×10^5 cells/75 cm^2 tissue culture flask. Cultures were allowed to attain approximately 90% confluency prior to experiment.

2.5. Western blotting

Western blotting was employed as described previously [20], to evaluate the expression of proteins. Briefly, SCs were cultured in serum free 1:1 DMEM and Ham's F12 medium with 60 mM glucose in the absence and presence of 10 nM insulin for 72 h. After the treatment, cells were washed thrice with Kreb's Ringer phosphate (KRP) buffer and incubated with 15 μg/ml heparin in KRP buffer for ten minutes. Later, cells were sonicated to prepare the protein lysate. Bradford's method was used to estimate the protein concentrations with BSA as a standard.

Protein (30 μg) from each sample was resolved on 12% SDS polyacrylamide gel. After electrophoresis, the proteins were transferred onto PVDF membranes (Millipore, India), blocked with 5% skimmed milk solution, and blotted with anti-LPL primary antibody overnight at 4 °C. Blots were then washed in tris buffered saline with 0.1% tween-20 and treated with secondary antibody conjugated with HRP, and developed with TMB/H_2O_2 substrate (Genei, India) to visualize the immunoreactions.

2.6. RNA isolation and real time PCR

Total RNA was isolated from Schwann cells after the time course of treatment with insulin, using total RNA isolation kit according to the manufacturer's protocol. mRNA expression studies were performed as described previously [18], by using StepOne plus real time PCR (Applied Biosystems, USA). The specific primer sets were used for both LPL (forward 5′-GAGATTTCTCTGTATGGCACA-3′; reverse 5′-CTGCAGATGAGAAACTTTCTC-3′), and RPL19 (forward 5′-CGTCCTCCGCTGTGGTAAA-3′; reverse 5′-AGTACCCTTCCTCTTCCC-TAT-3′). Data were processed using StepOne™ software v2.2.2. The fold change was calculated for the LPL mRNA expression by normalizing with RPL19 housekeeping gene using delta–delta Ct method ($2^{-\Delta\Delta Ct}$).

2.7. Immunocytochemistry

SCs cultured on glass cover slips were treated with 40 mM and 60 mM of glucose in the presence and absence of 10 nM insulin in serum free medium for 72 h. After treatment, cells were washed with PBS and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min, and further permeabilized by treating with 0.5% saponin for 15 min. Cells were then incubated with DPBS containing 10% FCS for 1 h to block the nonspecific binding sites. Anti-LPL antibody diluted in DPBS containing 5% FCS was then added and incubated overnight at 4° C. After washing with DPBS, cover slips were treated with goat anti-mouse FITC conjugated secondary antibody in dark at room temperature for 1 h. Finally, cover slips were rinsed with DPBS and mounted on glass slides. Immune reaction was visualized under a fluorescence microscope (Olympus, Japan) and photographed.

2.8. Immunohistochemistry

Immunohistochemistry was performed as described earlier [21]. Briefly, sciatic nerve tissues of control, diabetic and insulin treated diabetic rats were immersion fixed for 2 h in 2.5% glutaraldehyde in phosphate buffer, and then post fixed in freshly prepared 1% OsO_4 for 1 h. After dehydration, the specimens were infiltrated with the epoxy resin mixture and then entrenched in fresh epoxy resin mixture taken in capsules and polymerized in an oven for one day at 65 °C. Semi-thin sections of 0.5–1 μm were mounted on glass slides and stained with haematoxylin and eosin. Sections were examined under light microscope.

Please cite this article in press as: K.S. Rachana, et al., Insulin-induced upregulation of lipoprotein lipase in Schwann cells during diabetic peripheral neuropathy, Diab Met Syndr: Clin Res Rev (2018), https://doi.org/10.1016/j.dsx.2018.03.017