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Dietary restriction prevents dendritic changes of pyramidal neurons in hippocampal and prefrontal cortex in diabetic rat

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Abstract

Background and Objective: Diabetes mellitus (DM) is a common endocrine disorder with cognition decline. Dietary restriction (DR), also known as calorie restriction (CR), and reduction of foodstuff intake in rodents result in increased lifespan with protective potential. The aim of this study was to analyze the effect of DR on dendritic spine loss in streptozotocin (STZ) diabetic rats.

Materials and Methods: Rats were grouped into control, diabetic, and diabetic with DR. For induction of diabetes, STZ (*i.p.*) at a dose of 60 mg/kg was used. DR was applied from one month before till 2 months after diabetes induction. Finally, dendritic changes of pyramidal neurons in hippocampal and prefrontal cortex were assessed using Golgi impregnation method.

Results: Our histochemical findings showed a significant reduction of dendritic mushroom spines of pyramidal neurons of hippocampal CA1 and prefrontal areas in the diabetic rats that was significantly attenuated in the presence of DR.

Conclusion: DR application could appropriately prevent dendritic spine loss and in this way may have a beneficial effect on cognitive abilities in diabetic condition.

Keywords: Diabetes mellitus, Streptozotocin, Dietary restriction, Dendritic spines

1. Introduction

Dietary restriction (DR), also known as calorie restriction (CR), is regarded as one of the beneficial interventions that can extend life span in many animal species (1). DR could improve positive indexes of life quality including attenuation of weight gain, insulin sensitivity and beta cell function in some diabetic patients (2). In addition, DR has had protective effects against cancer and cardiovascular disorders (3, 4). Even, DR could decrease indices of anxiety in some behavioral tests in animal models (5).

Diabetes mellitus (DM) is associated with the generation of auto-antibodies against pancreatic β-cells (type 1 diabetes) or with development of insulin resistance (type 2 diabetes) (6). Global incidence of DM estimates more than 171 million for 2000 and 366 million for 2030 (7). The mortality and morbidity of DM are determined by various complications, such as diabetic vasculopathy, retinopathy, nephropathy, and peripheral neuropathy (8). Recently, many studies have indicated that DM also implicated the central nervous system (CNS) and induced the brain pathological changes (9). It has shown that DM induces a reduction in the spine density of apical dendrites of pyramidal neurons in two-month diabetic rats (10).

There is no evidence on the effect of DR in dendritic reorganization. Therefore, this study was designed to evaluate the beneficial effect of chronic DR on prevention of dendritic changes of pyramidal neurons

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in hippocampal and prefrontal cortex in STZ diabetic rats.

2. Materials and Methods

2.1. Experimental design

Male albino Wistar rats (from animal facility of Shaheed Beheshti Univ. Med. Sci., 9-11 weeks old, 185-225 g) were kept in an animal facility with access to food and water *ad libitum*. Used procedures were in compliance with NIH guidelines for the keeping and testing of laboratory animals. The rats (n = 24) were randomly divided into 3 groups, i.e. control, diabetic, and diabetic with DR. DR was applied from one month before till 2 months after diabetes induction defined as a 40% reduction in food intake. Diabetes was induced by a single injection of streptozotocin (STZ; 60 mg/kg; *i.p.*, Sigma-Aldrich, USA) (11, 12). After 10 days, blood was drawn from retro-orbital plexus under deep anesthesia with diethyl ether and serum glucose concentration was measured (glucose oxidation method, ParsAzmun, Tehran). Animals with a serum glucose level more than 250 mg/dl were selected for further experiments. Body weight was measured every week and serum glucose level was determined one week before STZ injection and on week 8 post-STZ.

2.2. Histochemical evaluation of dendritic spines using Golgi-impregnation method

After transcardial perfusion with normal saline containing heparin, right hippocampal tissue blocks (n = 4 from each group) were fixed in a solution of 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde. The used method for Golgi-impregnation method has been described in earlier studies (10, 13). After removing the brains, they were post-fixed, rinsed in phosphate buffer, and hippocampal blocks were incubated in a solution of 1% potassium dichromate, 1% mercury chloride, 0.8% potassium chromate, and 0.5% potassium tungstate in distilled water at room temperature for two weeks. After rinsing the brains with distilled water, they were incubated in a solution of 1% lithium hydroxide and 15% potassium nitrate in distilled water at room temperature for another two days. After tissue cryopreservation by their incubation in 30% sucrose and 0.1 M phosphate buffer for 1 day, hippocampal blocks were cut on a freezing microtome (Leica, Germany) at a thickness of 100 µm. The sections were transferred to gelatin-coated slides, dehydrated, and cleared with xylene, and immediately coverslipped with mounting medium. Number of mushroom spines were counted at a length of 50 µm on apical dendrites of pyramidal neurons of hippocampal CA1 area and/or prefrontal area. For each animal, spines were counted for at least 10 neurons and those cells that exhibited dark and consistent impregnation throughout the cell body and dendritic tree and its relative isolation from neighboring impregnated neurons were chosen for assessment. In addition, no primary dendrites were evaluated and all of the segments selected for analysis were located 100-150 µm far from the cell body and also not located at the end of the dendrite.

2.3. Statistical analysis

Data are shown as mean +/- SEM values. Parametric one-way ANOVA for other parameters were applied for analysis of data and if a significance difference was obtained, pair-wise comparisons were made using Tukey *post-hoc* test. In addition, $p < 0.05$ was considered significant.

3. Results

3.1. General findings

Body weight of diabetic group at 8^{th} week was significantly lower ($p<0.05$) as compared to baseline and DR application to diabetic group significantly attenuated the weight loss ($p<0.05$). In addition, with respect to serum glucose level, this parameter significantly raised in diabetic group at weeks 4 and 8 relative to baseline in diabetic group ($p<0.001$) and DR application to diabetic group significantly lowered serum glucose at weeks 4 and 8 ($p<0.01$) when compared to diabetic group at the same weeks.

3.2. Golgi-impregnation study of prefrontal and hippocampal CA1 areas

Light microscopic examination of Golgi-impregnated tissue of hippocampal CA1 area using Image Tool analysis software (version 3) showed a reliable and consistent neuronal staining. Tracing of the apical dendrites of pyramidal neurons from STZ-diabetic rats showed a significant reduction of dendritic mushroom-type spines versus control group ($p<0.01$) and DR application to diabetic rats significantly prevented dendritic spine reduction ($p<0.05$) (Figure 1).

Light microscopic examination of Golgi-impregnated tissue of medial prefrontal area also indicated a consistent neuronal staining. Tracing of the apical dendrites of pyramidal neurons from diabetic group displayed a significant reduction of dendritic mushroom-type spines versus control group ($p<0.001$) and DR application to diabetic group significantly prevented dendritic spine loss ($p<0.01$) (Figure 2).

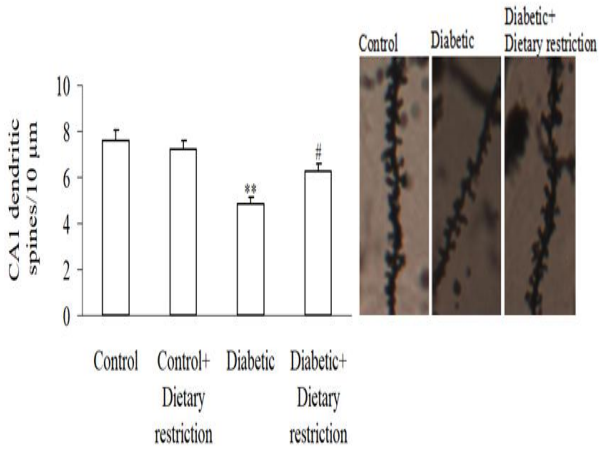

Figure 1. The mean spine density per selected length on the apical dendrites from pyramidal neurons of hippocampal CA1 area of different groups. ** p<0.01 (versus control), # p<0.05 (versus diabetic)

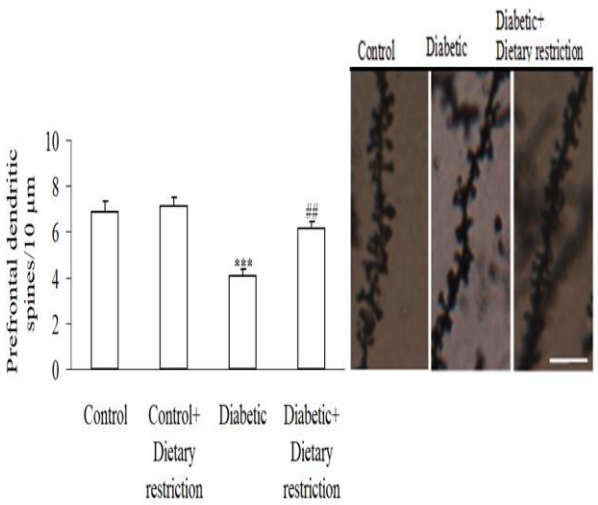

Figure 2. The mean spine density per selected length on the apical dendrites from pyramidal neurons of medial forebrain cortex of different groups. *** p<0.001 (versus control), ## p<0.01 (versus diabetic)

4. Discussion

The results of this study demonstrated that DR application for three months could appropriately prevent dendritic spines loss and in this way may have a beneficial effect on cognitive processes in STZ diabetic condition.

Although pronounced peripheral neuropathy is observed in patients with DM, the diabetic brain has not been studied so much and its dysfunctions still remain to be exactly determined. According to existing research evidence, patients who are diagnosed with Alzheimer's disease have a rather greater incidence of DM (14). However, no significant differences in the severity of Alzheimer-type pathologies such as senile plaques or neurofibrillary tangles have been observed between diabetic and normal brains (14). In addition, diabetics show impaired cognitive performance as compared to age-matched control individuals (15). Previous studies failed to find any changes in neuronal cell number using Nissl staining (16, 17). Using Golgi impregnation staining in our study, morphologic changes were evident in the CA1 and medial prefrontal areas of the cortex in the diabetic group two months after diabetes induction with STZ, as shown by lower densities of dendritic spines.

In this study, application of DR exerted a neuroprotective effect in diabetic group and decreased dendritic spine loss in CA1 and medial prefrontal areas of the cortex. On the other hand, application of DR for 3 months exerted an effect similar to a protective action, as shown by lower injury to dendritic spines due to induction of DM. In support of our findings, it has shown that DR could promote cell regeneration and stress resistance in various models of human disorders including neurodegenerative diseases such as Alzheimer's disease (18). In this respect, nutrient restriction could lower astroglial and microglial NF-κB activation in response to neurotoxic agents and is able to preserve astroglial autophagic flux and to lower intracellular accumulation of neurotoxicants (18). In recent years, attention has been toward DR as a potential neuroprotective strategy. Research evidence indicates that DR may prevent development of chronic disorders and could enhance longevity in different animal models (19), modulating energy metabolism, antioxidant defenses, inflammation and even autophagy (20). Moreover, DR can prevent age-related alterations in motor and cognitive function, dendritic plasticity and glial function in rodents (21, 22). In agreement with our findings, it has also claimed that application of chronic DR could suppress age-related inappropriate changes in dendritic spines (22). Part of beneficial effect of DR has been attributed to enhancing brain level of insulin-like growth factor-1 (23). Additionally, it has shown that chronic DR could prevent diabetogenic effect of STZ in mice, indicating that STZ might have been ineffective to exert its toxic actions on pancreatic beta cells by long-term DR (24). However, further researches are warranted to unravel the mechanisms of DR protection against diabetogenic action of STZ in experimental animals, especially its beneficial effect on the central nervous system under different toxic conditions.

In summary, chronic DR application could appropriately prevent dendritic spine loss and in this way may have a beneficial effect on cognitive abilities in diabetic condition.

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Conflict of interest

The authors declare no conflict of interest.

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