Original Article

Vitamin D Improves Learning and Memory Impairment in Streptozotocin-Induced Diabetic Mice

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Abstract

Introduction: Diabetes mellitus (DM) is associated with memory and learning deficits. Evidence has been provided that vitamin D is involved in brain function. The aim of the present study was to determine the potential effect of vitamin D on acquisition and retention of memory and learning in streptozotocin (STZ)-induced diabetic mice.

Methods: Experiments were performed in four groups of mice (each group; n = 7). Male mice were induced to diabetes by single dose (60 mg/kg, i.p.) injection of freshly prepared STZ dissolved in cold normal saline. Treatment with vitamin D (5μg/kg daily, i.p. dissolved in tween80) was begun at three days after diabetes induction. Passive avoidance (PA) learning method was used four weeks later. Retrieval test was carried out 24 h after training.

Results: Our results demonstrate significant impairment in acquisition and retrieval processes of PA learning in STZ- induced diabetic mice. Treatment with vitamin D improved learning and memory compared to the control group, both in acquisition and retrieval stages and reversed learning deficits in diabetic mice. In acquisition test, there were significant differences in the initial latency among the DM+Vit. D treated and control groups ($P < 0.05$). There was a significant difference in step-through latency between diabetic group treated with vitamin D compared to diabetic non-treated groups ($P < 0.05$).

Conclusion: It is possible that the effects of Vitamin D on cognitive deficits in STZ-induced diabetic mice could be mediated through calcium homeostasis modulation. These findings suggest a potential role for vitamin D in the treatment of diabetes-associated cognition deficits. The positive effect of vitamin D on the avoidance task may be attributed to its neuronal protective roles metabolic regulating roles of prolonged vitamin D administration.

Keywords: Diabetes, learning, memory, streptozotocin, STZ, vitamin D

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Introduction

Diabetes mellitus (DM) is a serious metabolic disease that can have deteriorating effects on various organs including the brain. Both types of diabetes play an important role in pathogenesis of brain diseases such as Alzheimer's disease.[1] Insulin and its receptors, which have been widely identified throughout the brain, are involved in various roles such as cognition and memory functions. In this regard, diverse evidence has demonstrated a decreased insulin level in CSF and impaired insulin signaling in patients with Alzheimer's disease.[2]

Both types of DM have been associated with reduced performance on cognitive function. But the cognitive dysfunction of diabetes is less addressed and has not been well recognized.[1,3] The issue of how diabetes can cause memory and learning system dysfunction is not clear. Duration-related cognitive impairment in diabetes is associated with increased apoptosis and decreased neuronal densities in rat diabetic hippocampi.[4] It is proposed that factors such as metabolic impairments, vascular complications and oxidative stress play possible important roles in pathogenesis of diabetic induced learning and memory deficits.[5]

It has been assumed that to be a two-way inter- relationship between DM and vitamin D difficulties. Recent evidence suggests that vitamin D insufficiency and polymorphisms of the vitamin D receptors (VDR) gene may affect the risk of type 1 diabetes.[6,7,8] However, new evidence has suggested that 1α, 25 dihydroxyvitamin D3 is involved in brain development and function.[8,9] It has been shown that the biosynthesis and degradative pathways of 1, 25-(OH) 2D3 exist in hippocampus and different areas of the brain. However, several evidences demonstrated the widespread presence of VDR and 1, α-hydroxylase in the developing and adult brain, and the known involvement of some vitamin D target gene products and its regulated processes in critical functions required for cognition and behavior. Thus, it is possible that vitamin D is involved in planning, processing and formation of memory and learning.[7,9,10,11]

Less attention has been given to the effect of diabetes and vitamin D on cognitive function. Although studies have indicated the role of vitamin D on glucose control and on improved diabetic state, its effect on the CNS remains to be elucidated.[7] Therefore, it is worthwhile to analyze the potential effects of long-term administration of vitamin D on memory dysfunction observed in STZ-induced diabetic mice.

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Materials and Methods

Subjects

Healthy male albino mice, 6–8 weeks old, weighing 20–25 g were used in current study. The Mice were maintained in groups of 5 per cage in a 12 h light-dark cycle at a controlled temperature (22 ± 1°C) with food and water ad libitum. All animal experimental procedures were performed following the guidelines of the National Institutes of Health and Animal Ethics Committee of Babol University of Medical Sciences.

The animals were divided into the following four groups: 1) Non-diabetic mice that did not receive any treatment; 2) STZ-induced diabetic mice that did not have any treatment (control); 3) Tween80-treated diabetic mice; and 4) vitamin D3-treated diabetic mice. Each group consisted of 7 animals.

Materials

STZ powder in analytical grade and Tween® 80 were purchased from Sigma-Aldrich (USA) and Merck (Germany), respectively. Vitamin D used in this study was donated by Osveh Pharmaceutical Company.

Induction of DM

Diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (60 mg/kg) dissolved in cold 0.9% saline solution in non-fasting mice.[12] Blood glucose was determined using an Accu-Chek system (Roche), and body weights were taken on a weekly basis for 4 weeks. Blood sample was obtained through a puncture in the tail. Diabetes in rats was confirmed on the third day by checking the fasting blood glucose concentration. Mice were considered to be diabetic when their non-fasted plasma glucose levels were ≥ 200 mg/dL.[13]

Diabetic mice were housed and treated with i.p. injection of 5µg/kg daily vitamin D dissolved in 0.3 mL Tween® 80 for 4 weeks by i.p. administration and then at the end of this period, learning studies were performed using a shuttle box or passive avoidance task.

Behavioral test

The study was carried out in a blinded fashion; i.e., the investigator was unaware of the study groups and type of treatment.

Apparatus

The behavioral study was done using PA task as a model of learning and memory.[14] The apparatus consists of a lighted compartment and a dark one. Between the two chambers, there was an opaque guillotine door. The floor of both chambers was made of stainless steel rods, but the floor of the dark part could be electrified.

Habituation

The habituation trials were performed for all experimental groups. For this purpose, the mice were placed in the lighted compartment of the apparatus facing away from the door and 5 s later, the guillotine door was raised. After the mouse entered the dark part, the door was closed and the animals were taken from the apparatus into their home cage.

Training

The mouse was placed in the lighted part facing away from the guillotine door and 5 s later the door was raised. After the rat entered the dark compartment, the door was closed and a 50 Hz square wave, 1 mA constant current shock was applied for 1 s. The mouse was retained in the apparatus and received a foot shock each time it reentered the dark part. Acquisition was terminated when the mouse remained in the illuminated part for 120 consecutive seconds.

In this trial, the number of trials (entries into the dark chamber) was recorded and the initial latency of entrance into the dark chamber was recorded.[15]

Retention

On the retrieval test that was given 24 h after the acquisition trial, each mouse was again placed in the illuminated part for retention study. The interval between placement in the illuminated chamber and entry into the dark compartment was measured as step-through latency (STL). The STL and the time spent in the dark compartment were noted as a measure of retention performance. The ceiling score was considered at 300 s and behavioral tests were performed at 8–12 h.

Statistical analysis

Data were analyzed using *t*-test and one way ANOVA *post-hoc* Tukey test. Results are expressed as mean ± SEM and the significance level was set at $P < 0.05$.

Results

Effects of vitamin D on acquisition of IA task

Figure 1 shows the initial latency to reach dark compartment in groups of non-diabetic, STZ-induced diabetic mice treated with i.p. injection of Vitamin D for 4 weeks and the diabetic group treated with tween80 in the same time. In acquisition test (Figure 1), there were significant differences in the initial latency among the DM+Vit. D treated (11.4 ± 5.2; n = 7) and control (29.12 ± 9.1; n = 7) groups ($P < 0.05$). The mean initial latency in the diabetic group treated with vitamin D was significantly different from the diabetic control group which did not have any treatment. Similar results were obtained in comparison of diabetic group treated with vitamin D and diabetic group treated with Tween80 (29.25 ± 7.3; n = 7) ($P < 0.01$). This finding reveals a positive effect of vitamin D on learning acquisition in STZ-induced diabetic mouse. The mean initial latency in the acquisition of passive avoidance task between non-diabetic and diabetic group treated with vitamin D did not show any significant difference ($P > 0.05$). In addition, the mean initial latency in the diabetic group treated with tween® 80 and the untreated diabetic group showed no significant difference ($P > 0.05$).

The results of PA task indicate that vitamin D had no effect on acquisition trials (Figure 2). The analysis of data of the groups shows that there is not any significant difference in the shock number of trials to acquisition criterion between vitamin D treated (3.8 ± 1.2) and control groups (2.65 ± 1, $P > 0.05$). One-way ANOVA test indicated that there was no significant difference in the number of acquisition trials in the four experimental groups ($P > 0.05$).

Effects of vitamin D on retrieval of IA task

In another experiment, retention test was performed 24 h later, which showed significant differences in the four groups. The vi-

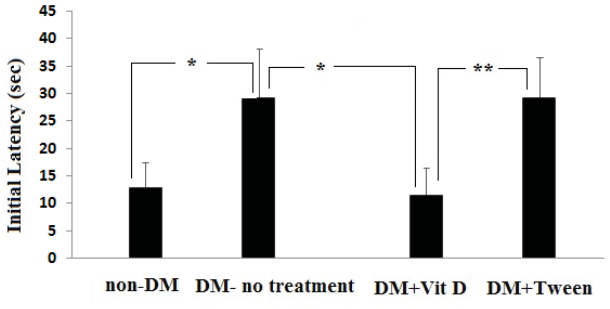

Figure 1. Initial latency in passive avoidance task for non-diabetic (non-DM), diabetic without treatment (DM- no treatment), Diabetic treated by Vit. D (DM+Vit D) and diabetic treated by Tween (DM+Tween) groups. Values are mean ± SEM (n = 7).* $P < 0.05$; **$P < 0.01$.

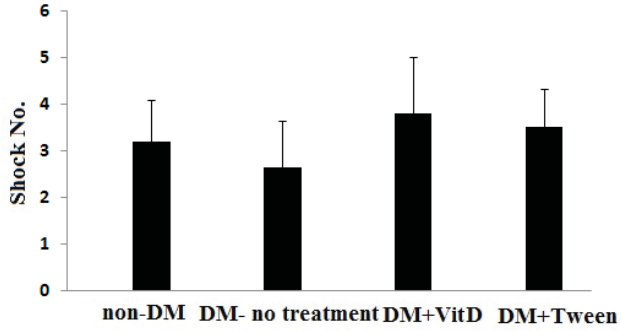

Figure 2. Shock number for acquisition of passive avoidance task in non-diabetic (non-DM), diabetic without treatment (DM- no treatment), Diabetic treated by Vit. D (DM+Vit D) and diabetic treated by Tween (DM+Tween) groups. Values are mean ± SEM (n = 7).

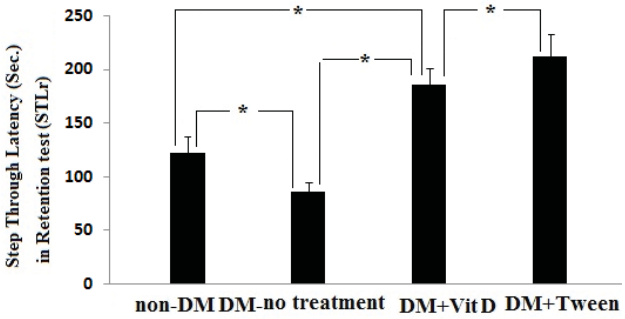

Figure 3. Step through Latency in retrieval test of passive avoidance task for non-diabetic (non-DM), diabetic without treatment (DM- no treatment), Diabetic treated by Vit. D (DM+Vit D) and diabetic treated by Tween (DM+Tween) groups. Values are mean ± SEM (n = 7). *$P < 0.05$.

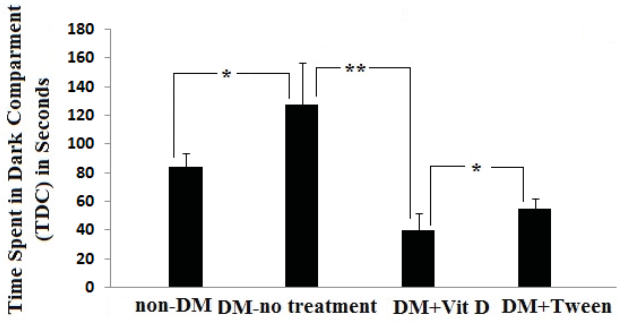

Figure 4. Time Spent in Dark Compartment (TDC) in retrieval test of passive avoidance task for non-diabetic (non-DM), diabetic without treatment (DM- no treatment), Diabetic treated by Vit. D (DM+Vit. D) and diabetic treated by Tween (DM+Tween) groups. Values are mean ± SEM (n = 7). *$P < 0.05$, **$P < 0.01$.

tamin D treated diabetic mice showed a significant increase in STL during 4 weeks of treatment compared to the control group. As shown in Figure 3, there was a significant difference in step-through latency between the diabetic group treated with chronic i.p. administration of 5 µg/kg daily vitamin D (185.2 ± 16.1) compared to the nondiabetic (122.7 ± 15.2; $P < 0.05$) and diabetic non-treated groups (86.5 ± 8.9; $P < 0.05$).

On the other hand, as represented in Figure 4, the results showed that the TDC in vitamin D treated diabetic group (39.7 ± 12.43) decreased significantly compared to the non-diabetic (84 ± 9.6; $P < 0.05$), diabetic non-treated groups (127.4 ± 28.9; n = 7; $P < 0.01$) and diabetic treated with Tween® 80 (54.8 ± 7.4; $P < 0.05$). Therefore, these results indicate that long term administration of vitamin D in STZ-induced diabetic mice significantly improves retrieval of PA task.

Discussion

The present study was done to determine the effects of chronic administration of vitamin D (i.p. injection of 5 µg/kg daily for 4 weeks after induction of diabetes) on the memory and learning disturbances caused by STZ-induced diabetes. The present study revealed that the STZ-induced diabetic mice show significantly reduced initial and retention latencies (STL), suggesting an impairment in acquisition and retrieval of learning and memory processes. Experimentally, it has been shown that STZ-diabetic rats could show impaired learning and memory functions.[16,17] However, damage to a variety of cognitive function domains has been reported in several studies but the pathophysiological details of cognitive dysfunction secondary to diabetes are not well understood.[16,18]

In this study, treatment of STZ-induced diabetic mice with vitamin D (5 µg/kg daily) for four weeks caused a significant improvement in both acquisition and retrieval of learning. The increased initial latency during acquisition, increased STLr and decreased TDC in retention test demonstrate positive influences of long term vitamin D administration on learning enhancement in STZ-induced diabetic mouse. In the retention experiments, decreased STLr and increased TDC of diabetic mice were reversed by vitamin D similar to the non-treated non-diabetic group. These findings are not in accordance with previous results showing that adult male rats were fed with vitamin D deficient diet for 6 weeks

prior to behavioral test. The group progressively learned to avoid the foot shock in active avoidance task and there was no difference in the latency to avoid the foot shock trials compared to the control.[19] Based on these reports, the positive effects of vitamin D on cognition have been proposed.[20] The previous studies have demonstrated the roles of vitamin D on learning[21] and behavior.[22] Similar to our results, a recent study has shown that the vitamin D-deprived rats had a significantly lower performance in Morris water Maze compared to both the controls and vitamin D3 receiving group. On the other hand, vitamin D supplementation did not significantly influence the learning in maze.[23]

The memory enhancing effects of vitamin D as nootropic agent on diabetic mice may be, at least in part, attributed to its physiological effects on Ca^{2+} homeostasis. It has been shown that brain calcium binding proteins are modulated by vitamin D. In addition to their calcium-buffering functions, they are required for normal signaling of evoked calcium transients in synapses and are involved in synaptic plasticity, long-term potentiation, and memory formation.[7,24,25]

The results observed in this study may be compatible with the antioxidant activity of vitamin D. Vitamin D3 acts as a membrane antioxidant through stabilizing the cell membrane against lipid peroxidation.[16] Evidence has been established for the role of free radicals and oxidative stress in complications associated with diabetes.[26] Implication of oxidative stress in the pathogenesis of DM is suggested not only by oxygen free radical generation but also due to impairment of antioxidant enzymes and the formation of peroxides.[27]

The brain is more sensitive than other tissues to oxidative stress because of its poorer enzymatic antioxidant defense mechanisms[28]; therefore, the significant increase in oxidative stress may have led to the significant cognitive dysfunction observed in our study. A previous study has shown that vitamin D3 treatment restores blood glucose homeostasis in streptozotocin (STZ)-induced diabetic rats.[29] Calgaroto, *et al.* reported significant physiological antioxidant activity for vitamin D.[16] Thus, vitamin D possibly acts by activation of antioxidant system and may create a proper condition for maintaining more appropriate brain functions.

To assess whether impaired PA performance of the diabetic mice is attributed to altered locomotion activity following diabetes or treatments, the locomotive activity was determined by activity monitoring apparatus (home made, Borj Sanaat) and their locomotion activity was recorded up to 15 min. We found that the number of locomotive activity at the end of 4 weeks was not significantly different in the studied groups. Therefore, the PA performance has not been influenced by locomotive changes.

In conclusion, current data suggest an important role for vitamin D3 enhancing effect on acquisition and retrieval of STZ-induced diabetic learning and memory impairment in PA task. Hence, more investigations are still required to obtain a more detailed comprehensive view of its action in cognition.

Competing of Interest

The authors declare that there are no conflicts of interest.

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