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Research Article

Effect of Magnesium Administration on Passive Avoidance Memory and Formalin-Induced Nociception in Diabetic Rats

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Abstract

Purpose: *To investigate the effect of oral consumption of magnesium on the memory and pain sensation of diabetic rats.*
Methods: *A total of 48 rats were divided into four groups - untreated control, untreated diabetic, magnesium-treated control and magnesium-treated diabetic. Plasma magnesium and glucose concentrations were measured after induction of diabetes with streptozotocin (STZ; 60 mg/kg). Four weeks after the administration of oral magnesium (10 g/L, $MgSO_4$), the animals were subjected to passive avoidance test whereby latency time (LT) was assessed. This was followed by formalin test which entailed the determination of licking and flinching scores*
Results: *Increased level of glucose and decreased concentration of magnesium in untreated diabetic group compared to untreated control group ($p < 0.001$) were observed. There was also a significant reduction in mean LT of untreated diabetic group ($p < 0.001$) as indicated by the increased number of animals that entered the dark compartment. Plasma glucose and magnesium levels in magnesium treated diabetic rats returned to normal 4 weeks after oral magnesium consumption. There was no significant change in mean total pain score despite elevated licking in diabetic animals after oral magnesium consumption. Significant elevation of flinching scores of untreated diabetic rats was observed in the last 20 min of the 2nd chronic phase, compared with the untreated control group.*
Conclusion: *It seems that magnesium treatment either restores rat memory performance that is impaired by diabetes or that it affects the aversive responses evoked by electrical shock.*

Keywords: *Diabetes, Magnesium, Glucose, Passive avoidance memory, Formalin test.*

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INTRODUCTION

There are several theories regarding the pathology and cognitive impairment in individuals with diabetes [1-2]. The pathways through which the disease affects on cognitive function are abnormality in glucose metabolism and formation of advanced glycation end products [3-4]. Thus, any change in glucose concentrations can affect cognitive function. From a mechanistic standpoint, it is difficult to discern whether the main mechanism relating type II diabetes to cognitive impairment is linked to glycemia, a component of the metabolic syndrome (e.g., hypertension), insulin resistance, or factors specifically related to adipose tissue. The answer could be that there is an aggregate effect of all the components on lifespan [5].

Occurrence of diabetes is a major risk factor for developing senile dementia which is regarded as one of the clinical signs of Alzheimer disease [6]. There are inadequate reports in the literature that associate diabetes and its effects on central nervous system and pain. However, one study has discussed the effect of diabetes on behavior, memory and learning [7]. Pain caused by peripheral nerve neuropathy is also one of the major clinical complaints described by patients with diabetes, and this affects the quality life of those concerned. Thus, alleviation of pain in these patients is of prime importance [8]. The results of a recent study have shown that high blood sugar is main cause~~s~~ of painful neuropathy caused by induced toxicity in peripheral nervous system [9].It seems that magnesium is a factor in the pathogenesis of diabetes as magnesium deficiency has been observed among 25 – 38 % of diabetic patients, in particular among those without an appropriate metabolic control [7]. This element modulates glucose transport across the membranes and is considered an important co-factor in enzymatic systems such as glucose oxidation [10]. The association between diabetes and low magnesium level is well understood and it is known that low magnesium diet leads to impaired insulin secretion and action [11] and that magnesium supplementation has been shown to prevent fructose-induced insulin resistance and lowers the incidence of type II diabetes [12,13]. In addition, the effect of oral consumption of magnesium on glucose control and improving insulin sensitivity is well-documented [14]. There is an inverse correlation between the magnesium absorption and diabetes and hence the diabetic patients are recommended to consume foods rich in magnesium such as cereals and green vegetables [15].

Therefore, the aim of this study was to assess memory performance and pain sensation in diabetic animals following consumption of oral magnesium using passive avoidance memory and formalin tests.

EXPERIMENTAL

Chemicals

Magnesium sulfate ($MgSO_4$) was provided from Merck Company and used as aqueous solution. Streptozotocin (STZ) was obtained from Sigma in lyophilized form ~~as~~ (1 g vials).

Animals and treatments

A total of 48 male rats locally produced in Naval Medical Research Institute (Razi, Co. Iran), weighing 180 - 250 g were used. The animals were kept in 12 h light/12 h dark cycles at 22 ± 0.5 °C and freely received water and their normal food. All the experiments were approved by the Ethical Review Committee (Project no. 5167, Qazvin University of Medical Sciences) and were carried out according to the Ethical Guidelines for the Investigations of Experimental Pain in Conscious Animals issued by the International Association for the Pain Study (16).

The animals were divided equally into 4 groups, as follows: normal control, diabetic, magnesium-treated normal, and magnesium-

treated diabetic ($MgSO_4$, 10 g/L of water). Oral magnesium administration in both magnesium-treated normal and diabetic groups continued for 4 weeks while the other two groups (normal control and diabetic groups) received water only. Induction of diabetes in diabetic groups was accomplished by a single intraperitoneal injection of STZ (60 mg/kg). One week after STZ injection, the animals with a minimum plasma glucose level of 250 mg/dl were considered as diabetic. The glucose concentration were measured with enzymatic-calorimetric kit (Zistshimi, Iran).Diabetic rats were given oral magnesium solution (10 g/L, $MgSO_4$) in their drinking water for four weeks.

Measurement of plasma magnesium and glucose levels

Development of diabetes following the administration of STZ was confirmed by measuring the blood glucose and magnesium level using the enzyme-based commercial kits(Zistshimi, Iran).. Plasma glucose and magnesium concentrations were measured spectrophotometrically (Rayleigh UV-2100 Spectrophotometer, China) at 520 and 510 nm wave lengths, respectively.

Passive-avoidance memory test

Using a shuttle box, passive-avoidance learning test was applied to measure memory and passive-avoidance response. The learning phase was carried out in four stages after a 5-min adaptation period in the shuttle box. A time interval of 1 h was considered between the stages of the learning phase, while the waiting time for each animal to enter the dark compartment was 5 min. Animals with LT higher than 5 min were excluded from the study. At the 4th stage of the learning phase and following arrival of the animal into the dark compartment, an electrical current of 1 mA intensity was applied to the animal footpads. The electrical shock was exerted within the last 5 seconds of the second minute using a shock generator instrument which caused the animal to jump and this was taken as proof of delivery of shock. Shortly after the delivery of shock, the guillotined door was opened to let the shocked animal enter the light compartment followed by removal of the animal from the instrument [17]. The delay time for animals to enter from the light to dark compartments was considered as latency time (LT). Once the 4th stage of learning was completed, data acquisition from the passive-avoidance test as a short-term performance criterion was carried out by determining LT at different time intervals (1, 6, 24 and 48 h,, and then 1 and 2 weeks) after shock induction. The passive-avoidance learning test was performed following magnesium treatment or induction of diabetes.

Measurement of pain scores (Formalin test)

Pain assessment was determined by Formalin test. This test was accomplished by injection of 50 µl of 2.5 % formalin solution as a chemical noxious stimulus into the animal planar hind paw followed by recording of the associated flinching and licking scores. The test was performed after the final session of passive-avoidance memory test.

Statistical analysis

Statistical analyses were performed using SPSS (version 16). Differences between the four groups were analyzed using Student t-test and one-way analysis of variance (ANOVA) while multiple comparisons between the groups was carried out with Tukey as post-hoc test. A probability value of < 0.05 was considered significant.

RESULTS

Effect of magnesium administration on plasma glucose

Mean serum glucose data are presented in Figure 1. Plasma glucose level at baseline and after 4 weeks for the untreated control

group was 107.0 ± 12.0 and 124.5 ± 6.6 mg/dl, respectively (p = 0.08). Similarly, the glucose concentration of untreated diabetic group at baseline and after 4 weeks was 478.0 ± 89.0 and 479.0 ± 63.0 mg/dl, respectively (p = 0.24). In magnesium-treated control group, plasma glucose concentration was 126.6 ± 18.7 mg/dl at the beginning of the learning phase and 119.0 ± 12.5 mg/dl at the end of 4 weeks (p = 0.13). Mean mean plasma glucose level in magnesium-treated diabetic group after 4 weeks was 140.5 ± 24.1 mg/dl and the difference between this group and the untreated diabetic group was significant (p < 0.001). There was no significant difference between the magnesium-treated diabetic and untreated control groups.

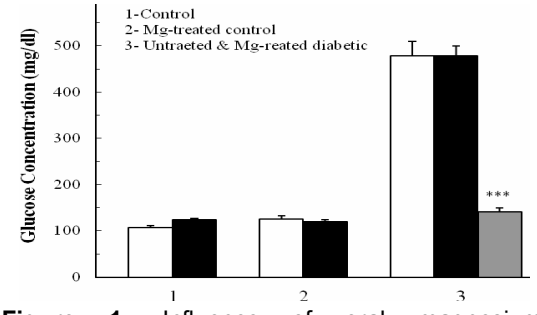

Figure 1: Influence of oral magnesium administration on plasma glucose concentration measured at time zero (transparent bars), after 4 weeks (dark bars), and 4 weeks following magnesium treatment (gray bars) in comparison with untreated diabetic rats; *p < 0.001.

Effect of magnesium administration on plasma magnesium

The plasma magnesium level of untreated control was significantly (p < 0.001) decreased more than that of the untreated diabetic group (2.47 ± 0.18 versus 1.65 ± 0.15 mg/dl). Furthermore, plasma magnesium concentration in magnesium-treated diabetic group (2.44 ± 0.23 mg/dl) significantly increased compared with untreated diabetic group after 4 weeks of magnesium treatment. However, there was no significant difference between the plasma magnesium levels of untreated control, magnesium-treated control, and untreated diabetic groups (p = 0.32).

Table 1: Latency time (LT) and frequency of animal entry into the dark compartment in passive avoidance memory test.

LT after test	Untreated Control	Untreated Diabetic	Magnesium-treated Control	Magnesium-treated Diabetic
1 h	162.0 ±2.8	47.7 ±1.9***	-	147.5± 2.4
6 h	158.0 ±1.8	45.0 ±4.9***	167.0±1.4	151.0 ±0.9
24 h	145.0± 0.8	38.0 ±5.7***	159.0 ±1.8	160.0± 2.4
48 h	135.0± 6.41	41.8 ±8.2***	152.1±4.0	152.5 ±2.4
1 week	122.7 ±10.0	35.0±7.6***	151.5± 9.3	120.0±18.4
2 week	112.0 ±11.3	36.2 ±10.5***	133.0±4.8	91.3± 9.4

*n = number of animals entered the dark compartment of shuttle box (mean ±SEM), n = 12); *= Statistical significance (p < 0.001) for latency time (LT) values, compared to untreated control group*

Effect of magnesium administration on passive avoidance memory

Evaluation of short-term memory was determined after 1, 6, 24 and 48 h, then 1 and 2 weeks following the 4th phase of learning and application of electrical shock. The results are presented in Table 1. Among the untreated diabetic group, mean LT was significantly lower than that of the untreated control group (p < 0.001). In addition, the frequency of entries in untreated diabetic control group was higher than those in untreated control group. Nevertheless, LT for both magnesium-treated control and magnesium-treated diabetic groups was insignificant compared to that of the untreated control.

Effect of magnesium administration on pain

The results of the evaluation of pain based on the formalin test (licking and flinching pain models) are depicted in Figure 2. The pain score for licking response, both during the acute (first 10 min) and chronic phases (15 - 60 min) for the magnesium-treated diabetic group was significantly (p < 0.05) than that for the untreated diabetic animals.

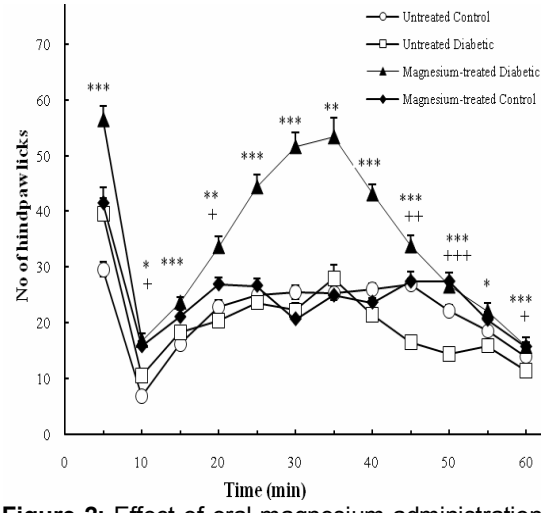

Figure 2: Effect of oral magnesium administration in formalin-induced biphasic pain model (Error bars denote SEM). ***Key:*** No. of hindpaw licks in untreated control (○), untreated diabetic (◇), magnesium-treated control (◆), and magnesium-treated diabetic (▲) groups; *= untreated diabetic in comparison with magnesium-treated diabetic groups; += untreated control in comparison with magnesium-treated control groups. *$p < 0.05$; **$p < 0.01$, and ***$p < 0.001$

Magnesium-treated control showed more licking than untreated control in the final 20 min of chronic pain phase (Figure 2).

However, in the flinching response pain model (Figure 3), significantly higher scores were recorded for untreated diabetic and magnesium-treated groups. The flinching scores of untreated diabetic group increased significantly, compared with untreated control group.

The total scores for both acute and chronic phases in magnesium-treated diabetic and magnesium-treated control groups were not significantly different from those of the untreated groups. However, as Figure 4 shows, the total scores for the chronic phase in the magnesium-treated diabetic and untreated diabetic groups were higher than those of magnesium-treated control and untreated control groups.

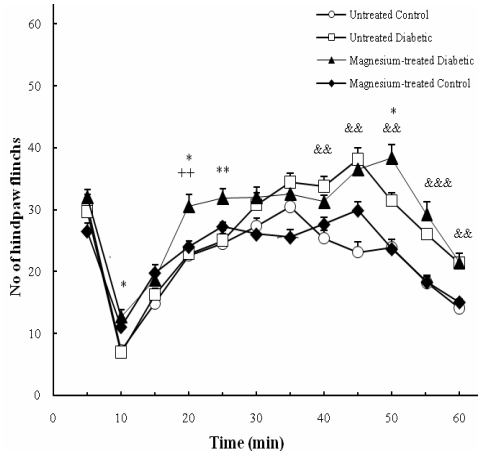

Figure 3: Effect of oral magnesium administration in formalin-induced biphasic pain model (Error bars denote SEM). No. of hindpaw flinches in untreated control (○), untreated diabetic (◇), magnesium-treated control (◆), and magnesium-treated diabetic groups (▲). *= untreated diabetic in comparison with magnesium-treated diabetic groups; += untreated control in comparison with magnesium-treated control groups; &= untreated diabetic in comparison with untreated control groups; *$p < 0.05$; **$p < 0.01$, and ***$p < 0.001$

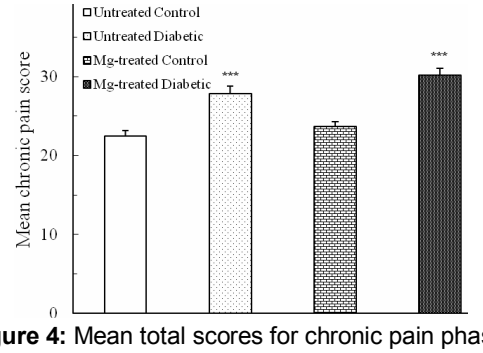

Figure 4: Mean total scores for chronic pain phase in four experimental groups. ***$p < 0.001$

DISCUSSION

Since total pain score in chronic pain phase was higher for untreated and magnesium-treated diabetic groups than for untreated and magnesium-treated control groups, it can be said that the hyperalgesia produced in the two diabetic groups was induced by elevated blood glucose. Furthermore, the aversive

response in magnesium-treated diabetic group; evoked by electrical shock, returned to its normal range. The higher LT values and the low number of animals entered the dark compartment for the magnesium-treated control group may be attributed to the increased sensitivity to electrical shock, leading to a mild alteration in aversive response. However, this assumption needs further investigation since the pain induced by formalin is due to a chemical noxious stimulus which is different from that induced by an electrical shock.

Magnesium reduction occurs in both type-1 and type-2 diabetes especially when toxins such as streptozotocin with known strong diabetogenic and pathologic effects (e.g., hypertension) are used [18-19]. Furthermore, it has been reported that continuous high serum glucose level is often accompanied by reduced magnesium serum level and that there is a correlation between magnesium deficiency, on the one hand, and insulin resistance and decreased insulin secretion in diabetic patients [20-21]. Many physicians have suggested daily administration of 300 - 400 mg of magnesium in diabetic patients having normal kidney function [15]. The restoration of plasma glucose concentration to the same level as that of control indicates that the mechanism is probably to that through which glucose transfer within membranes is modulated by magnesium and is considered an important cofactor in enzymatic systems such as glucose oxidation [14].

The present study revealed differences between the animal groups with regard to memory processing and function. Decrease in the latency time (LT) of the diabetic animals as well as the increase in the number of animals that entered the dark compartment are indicative of poor cognitive function induced by diabetes; this is consistent with earlier findings [1-2]. This is buttressed by entry of animals into the dark compartment 2 weeks later, indicating disappearance of memory associated with electrical shock. After four weeks of magnesium treatment, there was a significant decrease in the plasma glucose concentration and improvement in cognitive performance of the animals with experimentally-induced diabetes.

As reported by Obrosova, peripheral neuropathy is the most important and generalized complications of diabetic patients with a frequency of up to 60 % [22]. Neuropathic pain is a chronic pain manifested by a change in pain nociception, accelerated feeling of pain in response to painful stimuli (hyperalgesia), and abnormal painful sensitivity to those stimuli that were not painful earlier (allodynia) [23]. In an acute thermal test for pain processing, which involved measurement of pain threshold through tail flick latency, it was shown that oral magnesium administration prevents thermal hyperalgesia induced by diabetes in rats [24]. In agreement with our study, the findings of a recent report showed a significant reduction in plasma magnesium levels in diabetic animals. However, this condition was restored by 8-week oral magnesium administration, correcting the thermal pain threshold and plasma glucose level [24].

Formalin-induced pain behavior is considered a valid model of clinical pain (i.e., chronic pain experienced by man). The period of pain-induced behavior following subcutaneous injection of dilute formalin into rat hindpaw is divided into two phases, namely, an acute phase which is a temporary provocative response lasting approximately 2 - 5 min and a chronic phase which continues for at least 25 - 40 min, depending on the concentration of formalin used [25]. In the present study, the biphasic model of pain was clearly differentiated and recognized through licking and flinching modalities of pain. In this pain model, diabetic animals with no magnesium consumption showed an aggravated pain on both scores compared to control group, indicating the effects of neuropathy and hyperglycemia. In addition,

both diabetic and normal groups treated with magnesium for 4 weeks showeded hyperalgesia, unlike groups without magnesium treatment. It seems that the higher magnesium dose used in these animals is the likely cause of the hyperalgesia. It has been reported that an inverse correlation between magnesium absorption and diabetes exists, which is why diabetic patients are advised to consume foodstuffs rich in magnesium, such as cereals and green vegetables [15].

CONCLUSION

Based on the findings of the present study, it is clear that while oral administration of magnesium causes a significant decrease in plasma glucose, it considerably increases plasma magnesium levels in diabetic animals. Furthermore, long-term consumption of magnesium leads to the increased ability of diabetic animals to store information in memory space as well as increase their capacity to recall stored information.

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