The Effect of a Combination of Swim Training and Magnesium Supplementation on Thermal Hyperalgesia in Diabetic Rats

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Abstract

Objective: A question raised here as to whether exercise training combined with Mg supplementation could improve the symptoms of neuropathic pain such as the thermal hyperalgesia more effectively than either of the two alone. This study aimed to investigate the combined effect of swim training and Mg supplementation on thermal hyperalgesia in streptozotocin (STZ) induced diabetic rats.

Materials and Methods: Male wistar rats were randomly assigned to 5 groups: Control (C), Diabetic Control (CD), Training Diabetic (TD), Magnesium (Mg) Supplementing Diabetic (MgD), and Mg Supplementing and Training (MgTD). The training rats were subjected to forced-swimming for 8 weeks. Mg supplementing groups orally received magnesium sulfate (10 g/l) added into drinking water, for 8 weeks. Tail flick latency (TFL) test was used to evaluate the thermal pain threshold. Data were analyzed via multiple analyses of variance (MANOVA)

Results: Diabetes displayed the thermal hyperalgesia in most of diabetic groups at the late stage diabetes (P<0.05). Long term implementation of either swim training or Mg supplementation alone improved the severity of hyperalgesia (P<0.05). The combination of swim training and Mg administration prevented the occurrence of the hyperalgesia over the course of experiment (P<0.05).

Conclusion: These results suggested that the swim training go along with Mg administration could be a safe effective strategy for the prevention of painful diabetic neuropathy.

Keywords: Swim training, Mg supplementation, Thermal hyperalgesia, Streptozotocin-induced diabetes

Introduction

Diabetic peripheral polyneuropathy (DPN) is the most common type of diabetic neuropathy, with a prevalence as high as 27% in type 1 diabetes mellitus (T1DM) and 32% in type 2 diabetes mellitus (T2DM) (1). The main clinical symptom of DPN is the sensory loss, some DPN patients however experience hypersensitivity to stimuli known as painful diabetic neuropathy (PDN) (2). PDN is characterized by abnormal pain sensitivity to non-painful stimuli (allodynia) and a hypersensitivity to painful noxious stimuli (hyperalgesia) which is identified by a reduction of pain threshold (3,4). These complications seriously impair patient's quality of life (5).

Although many drug therapies are available for the treatment of PDN, some of them are not satisfactory, and some have undesirable side-effects (1,6). Hence, it is worthy to explore safe preventive strategies instead of drug therapies which might have side effects. It is well established that lifestyle modification included adopting a healthy diet and taking physical activity, can reduce the prevalence of diabetes and its complications such as diabetic neuropathy (7).

Dietary supplementation with micronutrients such as magnesium (Mg) has been known as a remedial strategy to prevent diabetic complications (8). On the other hand, Mg deficiency was reported in 25–30% and 13.5–47.7% in T1DM and T2DM, respectively (9,10) and contribute the pathogenesis of diabetic complications (11,12), such as diabetic neuropathy (13). In this respect, Mg administration can be a remedial approach in the management of diabetes-associated chronic neuropathic pain (5). Previous studies showed that Mg intake could prevent tactile and thermal allodynia, and could attenuate and delay mechanical hyperalgesia in the painful peripheral neuropathies of diabetic rats (5,14).

Exercise training was also recognized as an adjunctive therapeutic strategy to improve diabetes symptoms of neuropathic pain (15) including tactile, cutaneous and visceral hypersensitivity (15,16), mechanical allodynia (17) and thermal hyperalgesia (17,18). Nevertheless, exercise training has an unfavorable effect on Mg status (19,20). So, it has been observed to cause a mild reduction of Mg levels in plasma, mononuclear, erythrocyte, liver and muscle (21,22). Thus, a question raised here as to whether exercise training combined with Mg supplementation could improve the symptoms of neuropathic pain such as thermal hyperalgesia more effectively than either of the two alone. Therefore, the aim of this study was to investigate the combined effect of swim training and Mg supplementation on thermal hyperalgesia, in STZ-induced diabetic rats.

Materials and Methods

Forty five Male Wistar rats (8 weeks old and 210±20 gram weight) were purchased from Pasture Institute (Tehran, Iran). The animal experiment was approved by the institutional ethical committee of Bu-Ali Sina University (Hamedan, Iran).

All rats were housed, four rats per cage, in an animal room under a 12-h light-dark cycle at 22 ± 1°C and 60 ± 5.0 % humidity, and were provided with water and standard rat ad libitum. A 1-week period was assigned as adaptation period and the experiment was carried out after the period.

Rats were randomly assigned to 5 equal groups (number = 9): 1) Control (C), 2), Diabetic Control (CD), 3) Training Diabetic (TD), 4) Mg supplementing Diabetic (MgD), and 5) Mg supplementing and Training Diabetic (MgTD).

At the age of 9 weeks, a single dose of 60-mg/kg of body weight streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in a citrate buffer (0.1 mol/L, pH 4.5) was intraperitoneal injected to subjects of diabetic groups (D, TD, MgD, MgTD). 2 rats (1 from TD and 1 from MgD group) died as a result of injecting STZ. At 72 hours after the injection, animals with the fasting plasma glucose of ≥13.8 (mmol/l) obtained from orbital sinus were considered as diabetic subjects. Hence, 2 rats (1 from CD and 1 From MgTD group) were excluded. Finally, 8 rats from each group were considered as the study subjects.

The vehicle non-diabetic control rats (C), received an equal volume of injected prepared citrate buffer (0.1 mol/L, pH 4.5).

At the age of 10 weeks, one week after STZ injection, MgD, TD and MgTD groups were subjected to remedial intervention, including swim training and Mg administration for 9 weeks. The subjects of training groups (TD and MgTD) underwent to forced-swimming (5 days per week) in a pool (length 100 cm, width 100 cm, depth 50 cm) filled with tap water maintained at a constant temperature (37°C). The work load of training, the duration

of swim sessions, increased in 3 stages (each stage was 3 weeks). The duration of training at stages 1, 2 and 3 was 60, 90 and 120 minutes, respectively. A 3-day period (15 minute per day) was provided prior to training protocol in order that the rats would be adapted to swimming in the water pool. The non-training rats (C, D, MgD) were placed in shallow water (5 cm of depth), at 37°C water, 30 minutes each session, 5 sessions/week, for 8 weeks.

The subjects of Mg-supplemented groups (MgD and MgTD) orally received magnesium sulfate (10 g/l) added into drinking water (ad libitum). The subjects of non-supplementing groups (C, D, TD) only received tap water.

Body weights of all rats were measured weekly using digital weighing scales (accuracy of 0.01gr). However, just the body weights at 9^{th} (the onset of experiment), 10^{th} (pre-remedial intervention) and 19^{th} weeks of age (post-remedial intervention) were reported in this article.

By 72 hours after the injection of STZ (pre-remedial intervention) and 48 hours after termination of training and supplementation (post-remedial intervention), blood samples were taken in fasting state from orbital sinus using capillary glass tubes containing heparin under light ether anesthesia. Plasma was separated by centrifugation (10 minute, 3000 rpm, at 4°C). Plasma glucose level was determined by enzymatic method using glucose peroxidase enzyme kit (Zistshimi, Iran) and Hitachi autoanalyzer (902; Boehringer Mannheim, Germany). Plasma Mg level was measured by colorimetric method using an atomic absorption spectrophotometer (GBC Avanta, Australia) and a diagnostic kit (Pars Azmon, Iran).

Thermal sensitivity measurement

At 3th day and 4th, 7th and 10th weeks after the induction of diabetes which corresponded to 4th before and 3th, 6th and 9th weeks after the remedial intervention, respectively, the thermal pain threshold was measured by tail flick latency (TFL) test.

The TFL test was performed by a tail flick apparatus previously described by Dewey and Harris (1975) (23). Briefly, each rat was placed inside a restraining tube (length: 20 cm, diameter: 5 cm) in order to restrict body movement except the tail. The animal's tail was placed under a light source to expose the middle one-third of the tail to heat radiation. The time between light radiation and movement of the tail was considered as a tail flick latency, representing the thermal pain threshold. Each animal received 4 trials with a 10-minute delay between each trial (24). The light intensity of the apparatus was adjusted prior to the measurement in order that the mean of tail flick response would be approximately 4.0 seconds. The determined mean-intensity value was applied to all subsequent testing for all subjects (25). The cut-off time was set at 12 seconds to avoid damage to the tail.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Plot graphs were performed using excel software (Microsoft Office 2013). Statistically significant differences among multiple experimental groups on all measured data were determined via multiple analysis of variance (MANOVA) followed by a Tukey post-hoc analysis (IBM SPSS $Statistics_{23}$). A value of $P<0.05$ was considered as statistically significant.

Results

Body weight

As presented in table 1, the control group (vehicle-treated non-diabetic rats) showed an increase in body weight over the course of experiment. By comparison, the weight values of all diabetic groups (CD, TD, MgD, MgTD) were significantly lower, as compared to the age-matched control group at the end of experiment ($P<0.0001$). Further, the weights of the TD group were significantly greater than the diabetic control group, at post remedial intervention ($P<0.001$).

Table 1. Clinical Characteristic in the study groups before and after remedial intervention

Variable	C (n=8)	DC (n=8)	TD (n=8)	MgD (n=8)	MgTD (n=8)
Body weight (g)					
Pre-intervention	230 ± 4.39	228 ± 4.14	232 ± 4.50	234 ± 4.80	231 ± 5.1
Post-Intervention	300 ± 5.11	191 ± 7.22***	219 ± 6.23***†	188 ± 8.14***	205 ± 6.36***
Plasma Glucose (mmol/l)					
Pre-intervention	5.66 ± 1.16	25.0 ± 1.33***	23.8 ± 1.27***	25.5 ± 1.16***	24.4 ± 1.11***
Post-Intervention	5.5 ± 1.44	26.0 ± 1.61***	19.4 ± 1.4***†	18.8 ± 1.5***†	16.6 ± 1.5***††
Plasma Mg (mmol/l)					
Pre-intervention	5.96 ± 0.14	5.59 ± 0.17	5.66 ± 0.17	5.57 ± 0.14	5.71 ± 0.19
Post-Intervention	5.83 ± 0.17	3.64 ± 0.19**	3.28 ± 0.21**†	6.32 ± 0.21††	5.83 ± 0.19††

Data are presented as mean ± SEM. * indicates significant different compared to control group. †indicates significant different compared to diabetic group. *,†: P<0.05, ** , ††: P<0.001, ***,†††: P<0.0001 (MANOVA followed by Tukey post-hoc test). C: control, CD: diabetic control, TD: trained diabetic, MgD: mg supplemented diabetic, MgTD: Mg supplemented and trained groups, Mg: magnesium.

Plasma glucose

By 72 hours after STZ treatment, the fasting plasma glucose of most of the rats assigned to diabetic groups increased to above 13.8 mmol/l (32 from 34). Hereafter, these rats were considered as diabetic subjects. The elevated plasma glucose in the diabetic rats was statically different from that in the control group (P<0.0001).

The hyperglycemia in diabetic groups persisted till the end of experiment (P<0.0001). In comparison to CD group, fasting glycaemia values for the MgD, TD and MgTD groups were significantly lower at post-intervention (P<0.05).

Plasma Mg

As shown in table 1, plasma Mg levels in CD and TD groups were significantly lower compared with the control group at post-intervention (P<0.001). Likewise, the MgD, TD and MgTD groups displayed significantly higher plasma Mg levels as compared to CD group (P<0.05).

Thermal pain threshold

As shown in figure 1, on the basis of assessment at 3rd day and 4th week after STZ treatment, thermal pain threshold levels of all diabetic groups were not significantly different than control (P>0.05), implying the lack of incidence of the thermal hyperalgesia at early stages of STZ treatment. However, based on the assessment at 7th and 10th weeks after STZ treatment, the most of the diabetic groups including CD, TD and MgD displayed a significantly lower of thermal pain threshold value in comparison to age-matched control group (P<0.05), implying the occurrence of thermal hyperalgesia at late stages of STZ treatment. Interestingly, at all measurement occasions, the pain threshold values of MgTD group were not significantly different from that of age-matched control group (P>0.05), implying the absence of incidence of thermal hyperalgesia in this group over the course of experiment.

On the basis of assessment at 6th week of remedial intervention, the pain threshold levels of both MgD and TD groups were not significantly greater than the corresponding values for CD group (P>0.05), indicating the non-preventive effect of either training or Mg intervention alone on the emersion of hyperalgesia at this measurement occasion. However, on the basis of assessment at 9th week of the intervention, the pain threshold values of two groups were found to be significantly higher in comparison to age-matched CD group (P<0.05), indicating the relieving effect of either training or Mg intervention alone on the intensity of the hyperalgesia at this measurement occasion. In addition, the pain threshold values of MgTD group were observed to be significantly higher at all assessment occasions (P<0.05), indicating the preventive effect of combined training and Mg intervention on the appearance of hyperalgesia over the course of experiment. However, the pain threshold values of MgTD group were not significantly

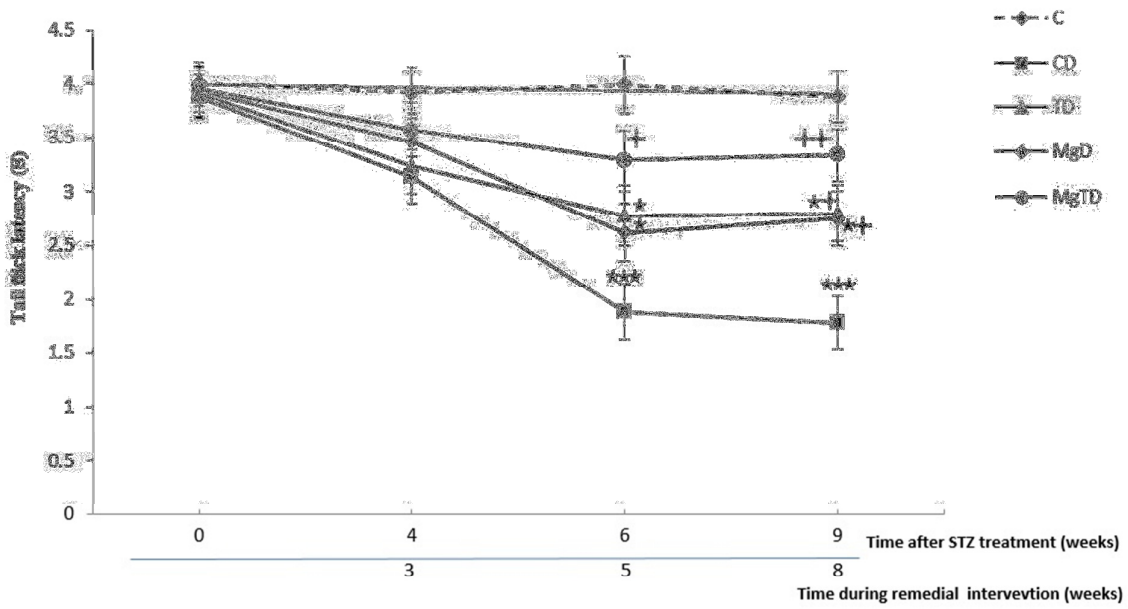

Fig. 1. Time courses of pain threshold in control (C), diabetic control (CD), trained diabetic (TD), Mg supplemented diabetic (MgD), Mg supplemented and trained (MgTD) groups. Data are presented as mean ± SEM. * indicates significant different compared to control group. $^{+}$ indicates significant different compared to diabetic group. *,$^{+}$: *P*<0.05, ** , $^{++}$: *P*<0.001, *,$^{+++}$: *P*<0.0001 (MANOVA followed by Tukey post-hoc test).**

greater than that of TD and MgD groups at all assessment occasions (*P*>0.05).

Discussion

The results of the present study showed that with 6 weeks of diabetes-induced STZ, a thermal hyperalgesia occurred. By long-term remedial interventions, i.e swim training or Mg administration alone, the severity of hyperalgesia was alleviated in the diabetic subjects. Interestingly, the combination of two interventions could significantly prevent the emersion of hyperalgesia over the course of experiment.

As expected, the results of this study showed that STZ-induced diabetes caused an attenuation of thermal pain threshold level in most of the diabetic groups (CD, TD, MgD) at latter stages of the measurement i.e 7th and 10th weeks following STZ treatment (Fig. 1). This indicated that the STZ-induced thermal hyperalgesia appeared at late stages of the STZ treatment. This is in agreement with previous studies who used TFL test to measure pain threshold and reported the occurrence of thermal hyperalgesia in STZ-induced diabetic rats and mice (25-27). Unlike this study, the STZ-induced thermal hyperalgesia in those studies was seen to occur rapidly (fewer than 14 days). However, Courteix et al. (1993), similar to this study, showed that the thermal hyperalgesia which was measured by tail immersion warm test occurred at late stage with STZ treatment (4 weeks)(28). This discrepancy appears to be related to different doses of STZ drug, to different settings of measurement devices, and to different occasions of measurement.

Chronic hyperglycemia has been demonstrated to strictly associate with peripheral nerve damages. These damages have been characterized by nerve dysfunction (29), by declined nerve conduction velocity (30) and by abnormally nociceptive behaviors, including tactile hypersensitivity (15) and thermal hyperalgesia (25). Add to these reports, our findings showed a coincidence of hyperalgesia and hyperglycemia in the diabetic subjects (Table 1). Nevertheless, no significant correlation was found between two factors. In

accord with our findings, some previous studies have suggested that the induction of the hyperalgesia is independent of the hyperglycemia (4,31).

The results showed that 9 weeks with swim training lessened the intensity of the thermal hyperalgesia in diabetic rats (Fig. 1). This finding has been similar to recently previous studies. Stagg et al. (2011) reported that five weeks of treadmill exercise training ameliorated thermal hypersensitivity in spinal nerve–ligated rats, a neuropathic pain model (32). Rossi et al. (2011) found that in STZ-induced diabetic female rats, forced-swimming for eight weeks reduced the thermal hyperalgesia (18). Chen et al. (2013) also showed that the progression of thermal hyperalgesia in STZ-induced diabetic rats was delayed by eight-week treadmill training (17).

Mechanisms thought to mediate the ameliorative effect of exercise training on the hyperalgesia may include: promoting of neurotrophic support, silencing of low-voltage activated (LVA) Ca^{2+} channels (15), increasing in heat shock protein 72 (Hsp72) expression (17), enhancing of brain-derived neurotrophic factor (BDNF) expression in CNS, and rising in endogenous opioid content (32). Based on some studies (15,17,32), the possibility of involvement of endogenous opioids in the exercise training-induced alleviation of hyperalgesia appears to be stronger than others. A reduction of basal endogenous opioid content contributing to an enhanced pain (33) has been observed in diabetic animals (34,35). On the other hand, aerobic exercise has been found to increase nociceptive threshold and reverse sensory hypersensitivity with concomitant rising of endogenous opioid content in the brainstem. Moreover, the anti-nociceptive effects of exercise training were restored by using systematically administered opioid receptor antagonists (naloxone) (32,36). Based on these evidences, we indirectly postulate that the improving effect of swim training on the thermal hyperalgesia may be, in main part, mediated by enhancing endogenous opioid content in CNS.

The current study showed that oral Mg supplementation for 9 weeks, like the effect of swim training, lessened the intensity of thermal hyperalgesia in the STZ-diabetic rats (Fig. 1). This result was consistent with previous studies reporting that Mg supplementation delayed and reversed the development of mechanical hypersensitivity (5,14). Similarly, Hasanein et al. (2006) have shown that 8 weeks of Mg administration could impede the thermal hyperalgesia in STZ-diabetic rats (37).

It is demonstrated that Mg deficiency could induce the diabetic neuropathy (13) and the hyperalgesia (38,39). Diabetes mellitus, in turn, has been suggested to lead to Mg depletion more likely by osmotic diuresis, resulting in the Mg deficiency (10,40). The present study also found that the STZ-induced diabetic rats displayed a mildly lower plasma Mg levels than the control group (Table 1), implying the occurrence of hypomagnesemia in the diabetic rats. However, the Mg supplementation could prevent from the Mg abnormality in the diabetic patients (Table 1). Taken together, the Mg intake seems to protect against the thermal hyperalgesia in the STZ-induced diabetic rats, at least in part, through prevention from hypomagnesemia which is implicated in nociceptive hypersensitivity.

N-methyl-d-aspartate (NMDA) receptor channels are evidently involved in central sensitization, leading to pain hypersensitivity (41-43). The Mg deficiency can facilitate NMDA receptor activation and thereby sensitize the nociceptive pathways (5,38). Hence, the blockade effect of Mg on NMDA receptors is thought to be, in part, an underlying mechanism by which Mg intake could relieve the intensity of thermal hyperalgesia in “the Mg supplemented diabetic rats”. Further, the Mg deficiency has been shown to cause an intracellular inositol depletion by reducing inositol transport rate, consequently provoking the diabetic neuropathy (44). Thus, it seems that the debarment of inositol depletion may be the other mechanism by which the Mg

administration could protect against the intensification of thermal hyperalgesia.

The main finding of present study was that the combination of swim training and Mg supplementation could prevent the occurrence of thermal hyperalgesia in STZ-induced diabetic subjects over the course of experiment. However, neither Mg intake nor swim training alone significantly impeded the development of the hyperalgesia; but they could alleviate the severity of hyperalgesia at long-term implementation (Fig. 1). These results suggest that the swim training combined with Mg administration could prevent the hyperalgesia at early stage of implementation through supplementing and augmenting each other's effects. Therefore, it could be suggested that healthy lifestyle change strategies including regular physical activity program and dietary Mg administration could safely and effectively prevent the painful diabetic neuropathy.

In this study, it was observed that the attenuations of pain threshold induced by swim training and Mg supplementation were coincident with the reduction of hyperglycemia in the diabetic subjects (Table 1). These prompted the possibility that the improving effect of swim training and Mg supplementation on the hyperalgesia may be, in part, related to the attenuation of hyperglycemia. However, no significant correlation was found between pain threshold and hyperglycemia value. This finding was consistent with previous studies showing that the improvements of hypersensitivity in diabetic subjects were independent of changing glycaemia (5,15,18,45).

The STZ-induced diabetic rats showed a marked reduction in weight gain. Moreover, the weights of the trained diabetic group were mildly greater than control diabetic one, suggesting the protective effect of swim training against the weight loss (Table 1). These results were consistent with previous studies showing that STZ-induced diabetes caused a body weight loss (4,28) and physical exercise mildly protected from the weight loss (17,46). These raised the possibility that the weight loss or the halt of weight gain caused by STZ-induced diabetes, reflecting a negative energy state, may be involved in the thermal hyperalgesia. However, in this study and another study (4) no statistical relations were found between the weight loss and the hyperalgesia.

Some limitations of the present study need to be considered. First, the results from this experiment in which rats were used as subjects would not be easily extrapolated to human, because the experimentally induced hyperalgesia could not completely reflect the diabetes-induced hyperalgesia in humans. Second, the sensitivity to thermal stimulus was only measured in this study, whereas the sensitivity to other stimuli such as the mechanic stimulus is also important in behavioral measurement of the painful diabetic neuropathy. Furthermore, besides TFL test, other thermal hyperalgesia test such as hot plate test could be used to measurement.

Conclusions

The results of the present study showed that long-term implementation of swim training or Mg administration was able to alleviate the severity of diabetes-induced thermal hyperalgesia. By comparison, the combination of swim training and Mg administration could prevent the occurrence of hyperalgesia over the course of experiment. These results suggest that life style modification by combined exercise training and Mg administration could provide a safe and effective strategy in order to prevent the painful diabetic neuropathy.

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