Lithium Decreases Streptozocin-Induced Diabetic Neuropathy in Rats by Inhibiting of Adenosine Triphosphate (ATP) Degradation

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Abstract- One of the most frequent complications of diabetes is diabetic peripheral neuropathy. Hyperglycemia would result in the advancement of this condition over a period of time. The most effective way in preventing diabetic neuropathy is regular control of glucose. In this study; we evaluated the effects of lithium onstreptozocin (STZ)-induced diabetic neuropathy in rats. Diabetic neuropathy was created 7 weeks after administration of STZ (45 mg/kg). Lithium was added to drinking water (450 mg/l) for 7 weeks and its plasma level after this period of time was 0.17±0.02 mmol/l. Levels of adenosine triphosphate (ATP) in dorsal root ganglion (DRG) neurons, oxidative stress parameters, open-field activity test and morphological analysis were assessed in this investigation. Currentresults showed significant elevation of oxidative stress biomarkers, reduction of ATP, abnormal morphology of DRG neurons and decrease of total distance moved in rats with STZ-induced diabetic neuropathy. The alterations in mentioned parameters were considerably restored by lithium treatment. These findings provide evidence for protective effects of lithium on STZ-induced diabetic neuropathy.

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Keywords: Diabetic peripheral neuropathy; Streptozocin; Lithium; Oxidative stress; Dorsal root ganglion neurons; Motor function

Introduction

Diabetes mellitus is a severe metabolic disorder that is increasing due to obesity population growth, aging, urbanization, and also socio-economic problems in recent years. Uncontrolled chronic hyperglycemia in diabetic patients leads to several complications including retinopathy, autonomic dysfunctions,and nephropathy. Over one-third of diabetics are suffering from diabetic neuropathy (1). Hyperglycemia and microvascular complications of diabetes stimulate the development of diabetic neuropathy (2).

Diabetic neuropathy is a debilitating disorder that is known by widespread damage to peripheral nerves. This disease is a progressive disorder that causes structural and functional changes in the peripheral and central nervous system. According to the complex etiology of diabetic neuropathy, various factors, including oxidative stress, hypoxia/ischemia of nerves, alterations in the protein kinase C pathway, decrease of nerve growth factor or even loss of advanced glycation end product (AGE) formation are involved in diabetic neuropathy disease (3-7). Although the cure for diabetic neuropathy is not well known, studies show that reduction of oxidative stress is effective in controlling of neuropathy. Therefore, the current study examined the effect of lithium as a free radicals' scavenger in Streptozotocin (STZ)-induced diabetic model in rats.

Lithium as a light alkali metal is not metabolized in the body and also cannot bind to the proteins (8). Some investigations show neuroprotective effects of lithium

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through various mechanisms like reducing expression of P53 and Bax as pro-apoptotic markers or enhancing the expression of Bcl2 as an anti-apoptotic factor, Ca^{2+} flux inhibition via NMDA receptors and inhibition of Glycogen Synthase Kinase 3beta (GSK3β) as a therapeutic target in central nervous system, cancer, and diabetes (9-11). Numerous factors containing glutamate excitotoxicity can induce activation of GSK-3 which is associated with apoptotic cell death (12). Direct and indirect inhibition of GSK-3 by lithium lead to increase expression of neuroprotective and neurotrophic proteins such as Heats Shock protein 70 (HSP70), Brain-derived neurotrophic factor and Bcl-2 (13). Deactivation of GSK-3 by lithium also stimulates cell survival transcription factors comprising cyclic adenosine monophosphate response element-binding protein (CREB), b-catenin, and activator protein-1 (13).

Initial attempts to treat diabetic neuropathy depend on glycemic control. However the role of lithium as a key player in ATP consumption, DRG histological alterations and oxidative stress was not studied completely in this disorder. In thepresent study, we evaluated the effects of lithium on STZ-induced diabetic neuropathy by assessment of lithium plasma level, ADP/ATP ratio, histological analysis of DRG neurons, and oxidative stress biomarkers.

Materials and Methods

Animals

Male Wistar rats with age 2-2.5 months and the weight of 200-250g were applied in this study and acquired from Tehran University of Medical Sciences; Faculty of Pharmacy. The rats were maintained in stainless cages and provided with free access to food and water. They were housed under adjusted lighting cycle (12/12hr light/dark cycle). All animal experimental procedures were carried out in accordance with Tehran University of Medical Sciences ethical guidelines for the use and care of laboratory animals.

Experiments

The animals were randomly divided into four groups containing (1) the control group that received intraperitoneal (i.p) injection of saline, (2) STZ group which received a single i.p. injection of STZ (45 mg/kg), (3) lithium-treated group which received lithium (450 mg/L, orally) in their drinking water for 7 weeks and (4) STZ/lithium group which treated with STZ (45 mg/kg, i.p.) and lithium chloride (450 mg/L, 7 weeks) via drinking water. Administration of lithium was simultaneous with STZ injection.

Induction of diabetic neuropathy

For induction of diabetes, 45 mg/kg of STZ dissolved in normal saline and administered i.p. to the rats with overnight fasting. One week after STZ injection, animals' blood glucose was measured. Animals with blood glucose of over 250 mg/dl were considered as diabetic. After two months, diabetic neuropathy occurred (14).

Determination of blood glucose level and body weight

Blood glucose levels and body weight of animals were determined respectively by using glucometer (Accu-chek-active) and special balance. We determined the blood glucose 72hrs and one week after injection of STZ to ensure the development of diabetes. Furthermore, we measured blood glucose finally, after twomonths, before surgery in animals.

Sample preparation

A mixture of xylazine (20 mg/kg) and ketamine (80 mg/kg) was administered intraperitoneally to anesthetize animals. About 5 ml blood was taken from the heart into the tube which contained heparin as an anticoagulant agent. Heart blood samples' were centrifuged at 4°c for 10 minutes at 1200rpm. Then plasma was kept at -80°c for evaluation of stress oxidative parameters.

For the purpose of tissue separation, the paraspinal tissue and spinal cord (C2 to L2) were removed. After laminectomy, the spinal ganglia were determined,and the sixth cervical DRG neurons on each side were detached. The spinal ganglia could be detected after removing cervical spine lamina. One or two separated DRG neurons were stabilized in formalin (10%),and the remaining DRG neurons were stored in liquid nitrogen for determining the ADP/ ATP ratio.

Measurement of plasma lithium concentration

The heart blood was collected in heparinized tubes just 7 weeks after induction of diabetes. The samples were centrifuged at 1200rpm,and plasma lithium level was determined by using a Shimadzu AA-670 Atomic absorption spectrophotometer.

Measurement of adenosine diphosphate (ADP) and ATP

All steps were carried out on the ice. The DRG neurons were taken from liquid nitrogen and sonicated in 250µl of ice-cold trichloroacetic acid (TCA, 6%). After centrifuging the extract for10 min at 12000rpm in

4°c, KOH (4M) was used to neutralize the supernatant and have the final pH of 6.5. Subsequently, it was filtered via 0.45µm Millipore mesh. ADP and ATP levels were measured in theneutralized extract by using Ion-Pair High-Performance Liquid Chromatography (IP-HPLC) (14).

Measurement of lipid peroxidation

The precipitate produced from mixing of plasma samples and TCA (20%) was dispersed in H_2SO_4 (0.05 M),andthiobarbituric acid (TBA) (0.2% in 2 M sodium sulfate) and consequently was added to the mixture. The samples were incubated for 30 minutes in a boiling water bath. The Lipid peroxide (LPO) adducts were elicited by addition of n-butanol,and the absorbance was measured at 532nm (15).

Measurement of plasma total thiol groups

The total amount of sulfhydryl in plasma was assessed as defined formerly. In the test tube, 0.2 ml of plasma was mixed with 0.6 ml of Tris-EDTA buffer (Tris base 0.25 M, EDTA 20mM, PH 8.2) and then 40 ml of DTNB (10mM) in methanol was added to the mixture. In order to reach the ultimate volume of 4.0 ml, 3.16ml of methanol was added. Afterwards, it was centrifuged at ambient temperature for 10 minutes at 3000rpm. The absorbance was assayed at 412nm (16).

Measurement of total antioxidant capacity

Antioxidant capacity of plasma was assessed by the ferric reducing ability of plasma (FRAP) test. By reducing ferric to ferrous, a blue colored complex of ferrous and tripyridyltriazine (TPTZ) with theabsorbance of 593nm was formed (14).

Measurement of reactive oxygen species (ROS)

In order to assay the activity of ROS, fluorescence DCHF was used as a probe with some modifications. The assay buffer included$MgCl_2$(5 mM), KCl (130 mM), NaH_2PO_4(20 mM), $FeCl_3$(0.1 mM), NADPH(0.1 mM), ADP (1.7 mM), Tris-HCl (20 mM) and glucose (30 mM) (PH=7.4, whole volume of 200 µL). In this test, theoxidation rate of DCFH to dichlorofluorescein (DCF), marker of oxidant generation, was determined at the excitation wavelength of 488 nm and emission wavelength of 525 nm, by means of ELISA F-2000 fluorescence spectrometer every 6 min for during thetime of 60 min (17).

Evaluation of open-field activity

Open-field activity test was performed to evaluate the effect of diabetic neuropathy on theexplorative behavior of rats. The rats were located in the test area and allowed to move freely for 15 minutes while all of their movements were recorded by a camera. The data were analyzed by Ethovision software for measurement of two parameters: distance moved and velocity (18).

Histological preparation and morphometric studies

Generally, DRG neurons were classified as a (large cells),and B type (small cells) cells by different definitions: cytoplasm of A-cells have a granular background with central nucleus, and also thelightly stained area around the cytoplasm of several cells can be seen in this type of cells. In B-cells, the homogeneity of cytoplasm and intensity of staining is more than A-cells. Central staining of cytoplasm in some B-cells is lighter than the outer parts.

Here, the tissues were embedded in paraffin after dehydration of DRG neurons in graded ethanol and clearing in xylol. Then they were sectioned in 40µm size by using a microtome. Haematoxylin and eosin were used to stain DRG neurons. The morphometric evaluation was performed by using HE-stained samples.

Olympus microscope (LX71, Japan) was used to count the number of A and B cells in all groups. Stereological studies were performed by image evaluation program (Optika, Italy) (18).

Chemicals

Streptozocin (STZ) was provided from Pharmacia and Upjohn Inc. (Kalamazoo, New Jersey, USA). Lithium, xylazine, ketamine, Adenosine diphosphate (ADP) sodium salt, KH_2PO_4 (analytical grade), ATP disodium salt, methanol (high performance liquid chromatography [HPLC]-grade, 5,5′-dithiobis-2-nitro benzoic acid (DTNB), 2-thiobarbituric acid (TBA), Tetrabutylammonium hydroxide (TBAHS), n-butanol, potassium hydroxide, trichloroacetic acid (TCA), diethyl ether, 2,4,6-tripyridyl-s-triazine (TPTZ), Tris-HCl buffer, 1,1,3,3-tetraethoxypropane (MDA), $MgCl_2$, Haematoxylin-eosin, formalin, xylol, alcohol, erythrosine, EDTA, KCL, $FeCl_3$ and NaH_2PO_4 were purchased from Merck (Frankfurt Germany). A SUPELCOSIL™ LC-18-T HPLC column was from SUPELCO (Antrim, UK), Nicotinamide adenine dinucleotide phosphate (NADPH), ADP, Dichlorodihydrofluorescein (DCFH) and bovine serum albumin (BSA) were supplied from Sigma–Aldrich (St. Louis, MO, USA).

Statistical analysis

The results are presented as mean±S.E.M. Statistical significance was determined by one-way analysis of variance (ANOVA) with post hoc test of Newman-Keuls. Differences with *P* of less than 0.05 were considered statistically significant. Graph Pad Prism 5 was used to analyze all obtained data from theexperiment.

Results

Assessment of body weight, blood glucose and plasma lithium levels in different treated animals

The body weight of animals was measured after 7 weeks. The results illustrated noticeable differences between different groups in this parameter (F=19.21, *P*<0.0001). Body weight of STZ group significantly reduced after 7 weeks in comparison with control and lithium-treated animals (*P*<0.001) (Figure 1). A significant increase in body weight in STZ/lithium combination group was observed as compared with STZ rats (*P*<0.001) (Figure 1). As shown in figure 1, there was no significant difference in body weight betweenthecontrol group and lithium-treated animals. We evaluated the blood glucose level 7 weeks after induction of diabetes by STZ. The results indicated significant differences between different groups in blood glucose concentration (F=51.09, *P*<0.0001). Blood glucose level in control and lithium group had an average of less than 110mg/dl .On the other hand blood glucose concentration in STZ and STZ/lithium group had an average of more than 250mg/dl.

The results related to plasma lithium level 7 weeks after lithium administration (450 mg/l) demonstrated notable differences between different groups (F=65.01, *P*<0.0001). The plasma levels of lithium in lithium and STZ/lithium-treated animals were 0.19±0.01 and 0.17±0.02 mmol/l respectively. In addition, the plasma concentrations of lithium in control and diabetic rats were 0.007±0.001 and 0.001±0.001 mmol/l. A significant difference in lithium concentration was observed between control and STZ with either lithium or STZ/lithium-treated animals (*P*<0.0001) (Table 1).

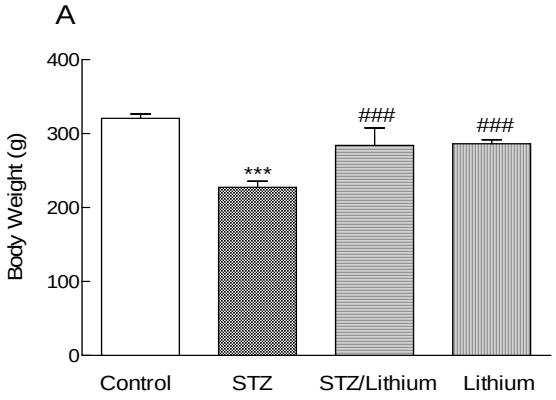

Figure 1. Effect of lithium administration on body weight

Significant reduction in body weight of STZ-induced diabetic neuropathy rats was seen in comparison with control animals (***P<0.001) and lithium group (###P<0.001). Body weight was significantly increased in STZ/lithium group comparing to STZ treated rats (###P<0.001). Data are represented as mean±S.E.M. of seven animals in each group. * and # show statistical difference between control and STZ treated animals respectively.

Table 1. Blood glucose and lithium plasma level in each group. A significant difference in lithium concentration was observed between control and STZ with either lithium or STZ/lithium-treated animals (*P*<0.0001).Data are represented as mean±S.E.M. Each group contains 7 animals

Treatment	Control	STZ	STZ/lithium	Lithium
Lithium plasma level (mmol/l)	0.007±0.001	0.001±0.001	0.17±0.02	0.19±0.01
Blood glucose (mg/dl)	98.258	434.667	353.33	100.429

Effects of lithium on STZ-induced motor dysfunction in neuropathic animals

In order to assess the motor function, we evaluated distance moved and velocity in the open field test 7 weeks after diabetes induction. The results indicated significant differences between different groups in the

distance moved (F=4.74, *P*=0.007). There was a significant reduction in distance moved in STZ group compared to the control (*P*<0.01) and lithium-treated animals (*P*<0.05). Treatment with lithium for 7 weeks significantly increased distance moved in STZ/lithium group compared with STZ group (*P*<0.05) (Figure 2). Additionally, the results demonstrated remarkable differences between different groups in velocity (F=4.10, *P*=0.01). Velocity in STZ rats was also decreased considerably in comparison with the control and lithium groups (*P*<0.05). A significant improvement of velocity was seen similarly in STZ/lithium-treated animals compared to STZ group (*P*<0.05) (Figure 3). As represented in figure 2 and figure 3, there was no statistical difference between control and lithium-treated rats in the distance moved and velocity.

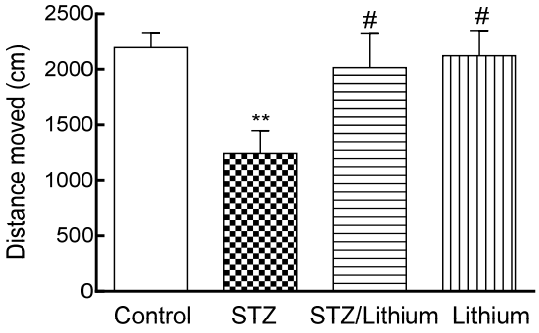

Figure 2. Effect of lithium administration on thedistance moved

The stz-induced diabetic group indicated considerable decrease in distance moved compared to the control (**P<0.01) and lithium group (#P<0.05). Lithium treatment in neuropathic group considerably increased the distance moved comparing to the STZ treated animals (#P<0.05). No remarkable difference was seen between control animals and lithium group. Data are represented as mean±S.E.M. of seven animals in each group. * and # show statistical difference between control and STZ treated animals, respectively.

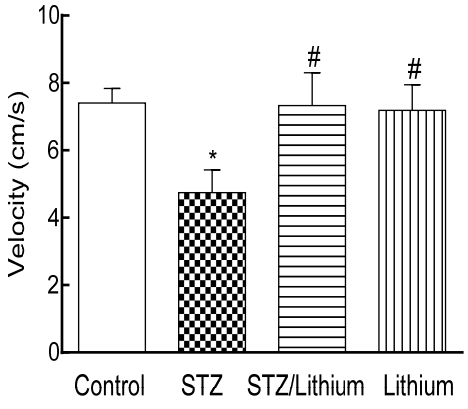

Figure 3. Effect of lithium administration on velocity

STZ treated animals showed significant decrease in velocity parameter as compared to control (*P<0.05) and lithium (#P<0.05) groups. The velocity was significantly increased in STZ/lithium animals comparing to STZ treated group (#P<0.05). No significant difference was seen between thecontrol group and lithium-treated rats. Data are represented as mean ± S.E.M. of seven animals in each group,* and # show significant difference with control and STZ treated animals, respectively

Assessment of oxidative stress biomarkers in control, STZ, lithium,and STZ/lithium-treated animals

The results showed notable differences between different groups in oxidative stress parameters (Thiol: F=43, *P*<0.0001, FRAP: F=3.88, *P*=0.03, LPO: F=13.16, *P*<0.0001, ROS: F=9.45, *P*=0.01).Intraperitoneal injection of STZ led to a remarkable decline of total antioxidant capacity (FRAP) (*P*<0.05) and thiol content (*P*<0.001) in plasma of STZ animals compared to the control group (Table 2).

Similarly a significant reduction of total antioxidant capacity (FRAP) (P<0.05) and thiol level (P<0.001) was also observed in STZ group compare to lithium-treated animals. The total thiol amount in STZ/lithium-treated animals was elevated remarkably as compared with STZ rats (P<0.001). Lipid peroxide (LPO) and reactive oxygen species (ROS) levels in plasma of STZ rats were significantly increased after 7 weeks of STZ injection in comparison with either control (P<0.001) or lithium (P<0.001) groups. There was a notable decrease in both LPO and ROS plasma levels in STZ/lithium treatment group compared to the STZ animals (P<0.001). Data showed no considerable differences in oxidative stress parameters between thecontrol group and lithium-treated rats (Table 2).

Table 2. Effects of lithium administration on different oxidative stress parameters

Treatment	Thiol (mmol/lit)	FRAP (mM)	LPO (µmol/ml)	ROS(u/mg protein)
Control	228.1± 5.72	88.9±4.50	303.3±17.44	2.87±0.13
STZ	121.3±4.95[a]	61.35±3.27[d]	407.6±9.88[a]	5.19±0.69[a]
STZ/lithium	188.4± 5.13[b,c]	75.23±1.26	318.1±13.16[b]	2.10± 0.57[b]
Lithium	209.4± 8.27[b]	80.17± 3.98[e]	297.74±13.55[b]	2.21± 0.12[b]

There was a significant change in plasma level of different markers in the diabetic neuropathy rats and the control group. Remarkable improving effects have been seen in STZ/lithium-treated animals comparing to the STZ treated group. Data are represented as mean ± S.E.M. Each group contained 7 animals. ([a]P<0.001 STZ,[c]P<0. and [d]P<0.05 the letters a, b and c are showing the comparison between the control group and other groups,[b]P<0.001 letter b shows the comparison between lithium and STZ/Li group with STZ group, [e]P<0.05 the letter e is showing the comparison between Li group and STZ group)

Analysis of ADP/ATP ratio in neurons of dorsal root ganglion

The result obtained from assessment of ADP/ATP ration in DRG revealed significant differences between different group (F=149.8, P<0.0001). Diabetes-induced by STZ resulted in a significant increase in the ADP/ATP ratio in dorsal root ganglion neurons of STZ rats in comparison with the control and lithium-treated animals (P<0.001). Lithium by increasing ATP level caused a significant attenuation of ADP/ATP ratio in STZ/lithium-treated rats compared to the animals which received STZ alone (P<0.001). No significant changes were seen between lithium-treated animals and control group (Figure 4).

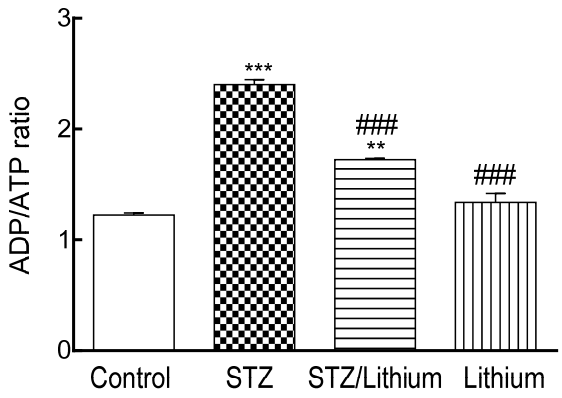

Figure 4. Effect of lithium administration on ADP/ATP ratio in dorsal root ganglion

There was a significant increase in ADP/ATP ratio in STZ-treated rats in comparison with the control (***P<0.001) and lithium group (###P<0.001). ADP/ATP ratio had a significant decrement in STZ/lithium-treated animals comparing to STZ treated rats (###P<0.001). A significant difference in ADP/ATP ratio was also seen between STZ/lithium-treated animals and the control group (**P<0.01). No considerable difference was observed between thecontrol group and lithium-treated rats. Data are represented as mean±S.E.M of seven animals in each group,* and # show significant difference with control and STZ treated animals, respectively.

Histological effects of lithium on the number of large (A) and small (B) cells

The result of Histological studies on A and B cells illustrated considerable differences between different groups (A cells: F=16.45, P<0.0001, B cells: F=5.86, P=0.006). There was a significant decline in the number

of large neurons (A cells) in STZ animals compared to control group (P<0.001) (Figure 5A). A considerable decrease was also seen in the number of large neurons in STZ/lithium-treated animal compare to control group (P<0.05) (Figure 5A). The number of large cells in STZ/lithium-treated group was significantly elevated as compared to STZ animals (P<0.05) (Figure 5A). The number of small cells (B cells) was significantly increased in STZ group in comparison with control rats (P<0.01). A remarkable decrease was also seen in the number of small cells (B cells) in STZ/lithium-treated animals compared with STZ group (P<0.05) (figure 5B). No significant difference was observed between lithium exposed animals and control group in the number of large and small cells (data not shown).

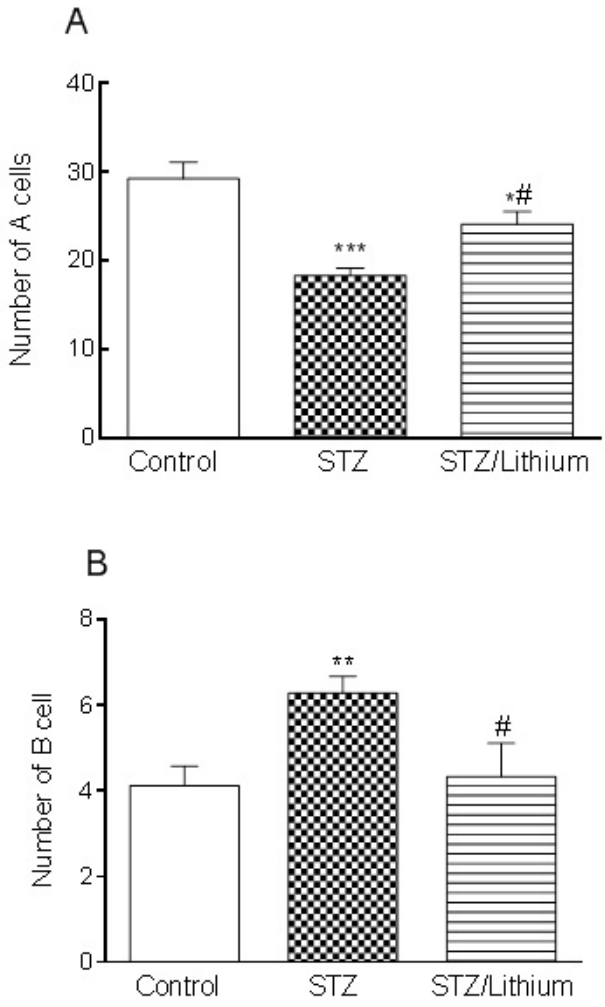

Figure 5. Effect of lithium administration on the number of large (A) and small (B) cells

A significant reduction in the number of A cells was observed between STZ treated animals and control group (A, ***P<0.001). Administration of lithium increased the number of A cells in STZ/lithium-treated rats compared to STZ group (A, #P<0.05). The number of B cells was considerably increased in STZ treated group comparing to the control rats (B, **P<0.01). The considerable decrease was observed in the number of B cells in STZ/lithium animals compared to STZ treated rats (B, #P<0.05). No significant difference was observed between control group and STZ/lithium group. Data are represented as mean±S.E.M of seven animals in each group. * and # show significant difference between control and STZ treated animals respectively.

Discussion

In the present study, we aimed to investigate the effects of lithium on STZ-induced diabetic neuropathy. Administration of lithium mainly improved oxidative stress parameters, motor function, and ADP/ATP ratio in a rat model of diabetic neuropathy.

In vitro and in vivo studies have revealed that there

are different biochemical pathways which are presumably involved in the progression of diabetic consequences, including polyol and hexosamine pathways, aberrant activity of protein kinase C and production of advanced glycation end products. Although each pathway may be harmful alone, they can result in instability state of mitochondria together, which leads to overproduction of ROS (2). Diabetic neuropathy induced by STZ was characterized by neuronal blood flow deficiency and also complications in nerve conductivity in diabetic rats (19). Deoxyglucose and nitrosourea as two segments of STZ are responsible for improving the ability of STZ cell permeability and toxicity of pancreatic β cells respectively that can cause an increase in blood glucose (20).

Vascular impairment followed by the high level of oxidative stress markers in diabetic rats results in endoneurial hypoxia and thereby reducing nerve conduction velocity and neuronal dysfunctions (20). High blood glucose concentration activates polyol pathway which reduces Na/K ATPase, myoinositol and NADPH activities. Reduction in NADPH activity prevents energy metabolism and subsequently attenuates the nerve conduction ability (20). Thus, the consequence of approximately all pathophysiological pathways involved in diabetic neuropathy is oxidative stress as the main cause of this condition (21).

Lithium, a monovalent cation, has been used to treat bipolar disorders for more than 60 years (22). It seems that plasma level of lithium has an important role in its biological effects. In our investigation, plasma lithium level of 0.17±0.02 mmol/l revealed anti-oxidant and protective effects on STZ-induced diabetic neuropathy. Chronic lithium therapy can prevent neuronal cell death and retardation of neurogenesis by enhancing protective markers and decreasing the pro-apoptotic biomarkers (11,23). Acquired results from our previous study in improving motor function and enhancement of nerve conduction velocity by administration of lithium in paclitaxel-induced neuropathy (24) show that one of the probable mechanisms of lithium is theprotection of neurons against hypoxia. In our investigation, no significant differences were seen in biochemical and behavioral parameters between lithium-treated animals and control group.

Diabetic neuropathy's in vitro model determines that high blood glucose suppresses regular mitochondrial function in DRG neurons (25). Augmentation of metabolic flux due to the high blood glucose motivates theexcess production of ROS (26). ROS are generated in electron transport chain of mitochondria and detoxified by glutathione, catalase, and superoxide dismutase as free radical scavengers. Overproduction of reactive oxygen species saturates capacity of the cell antioxidants which can lead to injuries of protein, lipid, and DNA (2,25). Recent study on oxidative stress and apoptosis induced by high glucose in PC12 cells indicated that lithium could decrease oxidative stress factors (27). Through long-term lithium therapy, increasing in glutathione concentration and preventing cell death mediated by H_2O_2 as reactive oxygen metabolite, were seen in primary cultured rat cerebral cortical cells (22). Various biochemical alterations have been assumed for neurotrophic properties of lithium against apoptosis and oxidative stress, including raise of the anti-apoptotic proteins, reduction of the pro-apoptotic biomarkers (22) and blocking of cytochrome C leakage (28). Also, it has been reported that high glucose-induced apoptosis was reduced by lithium administration in PC12 cells (27). Therefore, these consequences propose that the enhancement of anti-apoptotic factors caused by lithium can be partly considered as a probable mechanism of lithium in attenuating of diabetic neuropathy problems. Moreover, according to our finding that lithium reversed STZ-induced oxidative stress, thus it is possible that lithium by affecting ROS and other related pathways protect neurons against neuropathic situations.

In addition, analysis of ADP/ATP ratio of DRG indicated that this ratio increased in diabetic animals comparing to the control group. We showed a decline in this proportion after treatment with lithium. Apoptosis of neurons is developed by interruption of electron transport chain of mitochondria and ATP depletion. ATP depletion is caused by NADH agglomeration and also mitochondrial creatine phosphate pump failure in converting ADP to ATP (26). Even a slight decrease in the function of mitochondria and subsequent discharge of ATP may have noticeable effects on theperipheral nervous system (1). According to the results obtained in this study, it can be expected that lithium by reducing oxidative stress can improve the performance of mitochondria and thereby prevent energy depletion. The effect of lithium on insulin/glucose modulation is also not negligible (31). It is possible that lithium-induced oxidative stress inhibition and preventing of ATP depletion affect insulin/glucose functional attitude which needs to be clarified by details in future studies.

Elevation of oxidative stress and mitochondrial dysfunction are involved in the pathogenesis of neurodegenerative diseases such as Alzheimer disease (AD), Parkinson, diabetic neuropathy (29) and Amyotrophic lateral sclerosis (ALS) (30).

Neuroprotection mechanisms of lithium that proposed by recent evidence are improvement of mitochondrial dysfunction, reduction of oxidative stress, and protection against DNA damage caused by oxidative stress (30).

Development of oxidative stress coupled with thedischarge of ATP within apoptotic process can induce Na/K pump dysfunction (32). Reduction of Na/K ATPase activity may be the result of ATP depletion and seems to have a significant role in the pathophysiology of diabetic neuropathy (1). Several studies have shown that decreasing of Na/K ATPase activity in neuronal tissues can induce the progress of diabetic neuropathy (33). A number of studies have also confirmed that lithium could enhance Na/K ATPase activity of erythrocytes in manic-depressive patients (34). Furthermore, thesubstantial relevance between enzyme activity and lithium level has been detected (34). Research on animal models of ALS treated with lithium has shown considerable improvement in motor function. The principal assumption mechanism of lithium for such improvement was motivating autophagy (30). Since our data also represent the improvement of velocity in diabetic neuropathy rats, further studies need to be performedto determine the involved mechanism.

Diabetic rat neurons have shown different characteristics including smaller cells, more vacuoles with larger sizes and stronger basophilic staining (18). Slowing of nerve conduction velocity is because of losing large neurons (35). Although thetotal count of large and small cells are approximately equal in diabetic neuropathy and control group, large to small neurons ratio decrease under diabetic neuropathy condition (18). Regarding our results which show considerable increase of large cells and also significant reduction of small cells in Li/STZ treated animals and STZ group, it is convincible to consider lithium as a protective cause against STZ-induced histological alterations in neuropathic rats. Moreover, in vitro cell/cell line studies of pancreatic cells and dorsal root ganglion would contribute to understanding the mechanisms underlying an eventual protective effect of lithium against diabetic neuropathy which should have done in near future studies.

As a conclusion, present findings demonstrate beneficial effect of the treatment with lithium in diabetic neuropathy rats by enhancing motor function, increasing ATP, improving stress oxidative parameters, and refining of histological alteration of DRG. However, more investigations are needed to clarify the other interfering mechanisms and evaluate whether lithium could have place in the preventing of diabetic neuropathy in clinic.

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