Effects of Lithium on Peripheral Neuropathy Induced by Vincristine in Rats

Houman Alimoradi[1], Nasir Pourmohammadi[2], Shahram Ejtemaei Mehr[2], Gholamreza Hassanzadeh[3], Mohammad Reza Hadian[4], Mohammad Sharifzadeh[5], Azam Bakhtiarian[2], and Ahmad Reza Dehpour[2]

[1] Toxicology Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Anatomy, Tehran University of Medical Sciences, Tehran, Iran

[4] Brain and Spinal Injury Repair Research center, Tehran University of Medical Sciences, Tehran, Iran

[5] Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

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Abstract- Vincristine (VCR) as a frequently used antimitotic agent which is commonly prescribed for wide spectrum of neoplasm, causes mixed sensorimotor neuropathy. Several evidences show lithium could be a neuroprotective agent, therefore to assess whether a pretreatment and at subtherapeutic dose it could prevent the peripheral neuropathy produced by VCR, rats were treated with VCR 0.1mg/kg i.p. for 3 alternative doses and / or lithium chloride (20mg/kg or 40 mg/kg i.p. daily from the first day to the day of sacrifice). Erythrocyte lithium concentration (ELC) and plasma lithium concentration (PLC) were measured at the seventh day of study and the day of scarification. After seventh day of lithium administration, PLC and ELC reached to a steady state at subtheraputic dose and they did not significantly change at normal housing situation. Hot plate, open field test and nerve conduction velocity were used to evaluate the sensory and motor neuropathy. Only VCR treated rats showed behavioral, electrophysiological and histological evidences of a mixed sensorimotor neuropathy by significant increase in hot plate latencies and a marked decrease in total distance moved and conduction velocities in both sensory and motor nerves. Lithium at the dose of 20mg/kg and specially 40mg/kg robustly reduced the rate of mortality, general toxicity and was able to ameliorate mixed sensorimotor neuropathy induced by VCR. These results suggest that lithium at dose of 20mg/kg and 40 mg/kg, potentially by its effects on cell survival pathways such as inhibition of glycogen synthase kinase-3 (GSK3β), can prevent both motor and sensory components of VCR neuropathy.

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Keywords: Vincristine; Lithium chloride; Rat; Peripheral neuropathy

Introduction

VCR is a member of Vinca alkaloid family and is indicated for the treatment of Hodgkin, non Hodgkin's lymphoma and particularly in pediatric cancers either by itself or in combination with other antitumor agents (1,2). The cytotoxicity induced by VCR is based on well established pharmacologic properties that include, binding to tubulins and disrupting microtubules formation in mitotic spindles and thus preventing cell division (3,4). Lithium which is commonly prescribed for bipolar disorders, showed some neuroprotective effects in central nervous system (CNS) and these effects might be via interaction of NMDA (n-methyl d-aspartate) receptors, reduction in pro apoptotic proteins, p53 and bax, increased Bcl2, cytoprotective proteins and cell survival kinase activation (5,6). We have previously shown that lithium is able to ameliorate sensory neuropathy related to paclitaxel (7). Although Petrini *et al.*, has previously shown that lithium at therapeutic dose is able to ameliorate VCR induced neuropathy (8), their interventions were just two behavioral test (hot plate and cold test) and also VCR dose was much more than its LD_{50}. Moreover they did not measure the lithium level in serum or any pathologic changes in peripheral nerves. Here we designed a comprehensive study to show the potential neuroprotective effects of lithium at subtherapeutic doses and as a pretreatment on peripheral sensorymotor neuropathy induced by VCR.

Corresponding Author. Ahmad Reza Dehpour
Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel.: +98 21 88973652, Fax: +98 21 66402569,E-mail: dehpour@yahoo.com

Materials and Methods

Animals

Experiments were performed on 250 ± 30g male Sprague Dawley rats in pharmacology department of Tehran University of medical sciences. Animals were housed in a temperature and humidity controlled environment with 12 hr light/dark cycle. Food consists of normal rat chow and water ad libitum. This study was performed according to the guidelines of the US national institute of health (NIH publication no.85.23, revised 1985) guides for the care of lab animals. Rats were randomly divided into six study groups: (1) 12 rats received VCR 3mg/kg (VCR group); (2) 12 rats received VCR 3mg/kg and lithium chloride 20mg/kg (VCR+ Li 20 group); (3) 12 rats received VCR 3mg/kg and lithium chloride 40mg/kg (VCR+ Li 40 group); (4) 14 rats received lithium chloride 20mg/kg (Li 20 group); (5) 14 rats received lithium chloride 40mg/kg (Li 40 group) and finally (6) 8 rats received saline in the same program as VCR treating animals (saline group).

Drug administration

Vincristine sulfate (Sigma Chemical Co, St. Louis, MO) dissolved in saline and was injected intraperitoneally (*i.p.*) on three alternative days at the dose of 0.1mg/kg (total cumulative dose =0.3mg/kg). Lithium receiving groups took one intraperitoneal injection of Lithium chloride (Merck, Darmstadt dissolved in saline) in each day from the day of beginning to the day of sacrifice at two doses of 20mg/kg or 40mg/kg.

Survival study and general toxicity

To assess the general condition of animals, they were daily observed to compare signs of edema, cachexia, alopecia and mortality in different groups.

Measurement of lithium in plasma and RBC

At the 7^{th} day of study 6 rats from lithium receiving groups were randomly chosen and anesthetized by Pentobarbital sodium (65mg/kg) and about 6cc blood were collected from left ventricle. Blood samples were centrifuged at 1600g and then PLC and ELC were determined by atomic absorption spectrophotometer (GBC scientific pty ltd) as previously reported (9). The same measurement was done for all rats in lithium receiving groups before sacrifice.

Behavioral examinations

The effect of VCR on neuropathic pain and sensory neuropathy was evaluated by hot plate test two times per week from the first VCR injection to the last one. Animals (8 in each group) were placed on a 52±0.2c heated plate (Socrel hot-plate model DS37, Ugo Basile, Italy) and time spent until the first episode of heat sensitivity was including jumping, fore paw or hind paw licking (10). Motor impairment was evaluated by open field activity. Rats were placed into an area (diameter= 1.4 m) and locomotion within the area was tracked over a 10 minuets using a high resolution monochrome camera and stored and analyzed with ethovision software (v.8) and total distance moved (cm) was calculated (11,12).

Electrophysiological examination

After the last behavioral tests, to measure NCV animals were anesthetized with pentobarbital sodium (65mg/kg), body temperature was monitored and maintained within normal limits and then motor nerve conduction velocity (NCV) in the left sciatic was recorded as previously described using power lab (MLT 1030/D, AD Instruments, Power Lab, Spain) and the same stimulating and recording pin electrodes (AD Instrument, pin) (13).

Histological and morphometric studies

After a deep general anesthesia by sodium pentobarbital overdose (100 mg/kg *i.p.*), right sciatic nerve was approached and fixed in 1.25% glutaral-dehyde-1% paraformaldehyde in 0.2 M phosphate buffer at pH 7.4. Laminectomy was then performed and the lumbar (L4-L5) DRG (dorsal root ganglion) was exposed and fixed in the same fixator. Semithin (0.5µm) cross section of neurons were stained with hematoxylin and eosin (H and E) and were used for morphometric analysis (3 section for each animal were randomly observed), the following measurements were obtained: large cell (called A type) and small cell (called B type) diameters from DRG and G-ratio (it is defined as the ratio of the inner diameter to the outer diameter of a myelinated axon in sciatic sections) from sciatic sections.

Statistical analysis

The results are reported as mean ± S.EM. The statistical analyses were performed using one way analysis of variance (ANOVA) by SPSS (v.18). Group differences were calculated by post hoc analysis using Tukey test. For all tests, differences with values of $P < 0.05$ were considered significant.

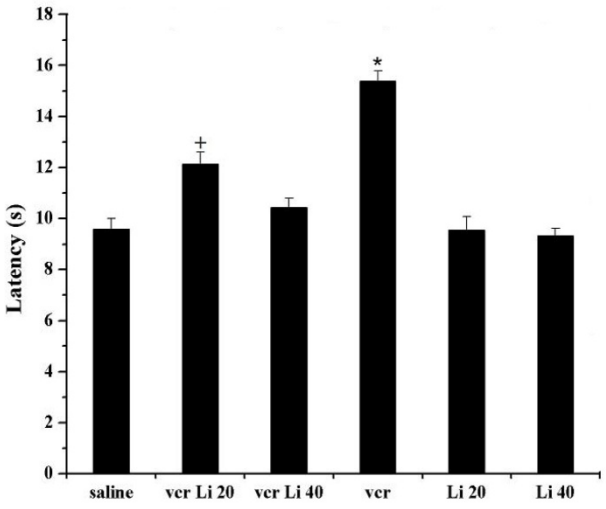

Figure 1. Hotplate response of rats treated with VCR with or without Li at doses of 20/kg or 40mg/kg. Animals treated only with VCR showed hyporalgesia after the last injection of VCR because withdrawal latency was significantly increased compare with saline (**P*<0.001). A significant decrease in latencies was observed between single VCR treated rats and VCR+Li 20 group (+*P*<0.05) and also no significant differences in latencies were detected between saline and VCR+ Li 40 group (*P*>0.05).

Results

Survival study and general toxicity

The rate of mortality in VCR only treated rats was around 41.6% and some marked signs of general toxicity such as looseness and decreased in amount of stool, alopecia, abnormal posture (consisting of foot and head drop) and a significant decrease in body weight (*P*<0.05) were observed in these animals compared with saline group.

Lithium at the dose of 20 and 40 mg/kg improved general condition of animals treated with VCR since fewer symptoms that above mentioned were observed in VCR+Li 20 group and VCR+ Li 40 group. In addition no significant body weight changes were determined in VCR+Li 20 group or VCR+Li 40 group versus saline group (*P*>0.05).

Plasma and erythrocyte lithium concentrations

PLC and ELC at 7th day of study reached to a steady state and these concentrations for lithium administration at the dose of 20mg/kg (n=6) were: PLC=0.236±0.006mM and ELC=0.137±0.005mM and for Li 40mg/kg (n=6): PLC=0.458±0.007mM and ELC=0.304±0.009mM. No significant differences in PLC and ELC were determined between the 7th day of study and the day of sacrifice (*P*>0.05).

Behavioral studies

A day after the last injection of VCR a significant thermal hypoalgesia , determined by increase in hotplate latency, was shown in rats treated with only VCR compared with saline group (P<0.001). Although hotplate latencies in VCR+ Li 20 group significantly decreased versus VCR group (*P*<0.05), thermal hypoalgesia still was observed compared with saline group (*P*<0.05). No significant differences in latencies were detected VCR+Li 40 group versus saline (*P*>0.05; Figure 1). Animals treated with VCR were infected with motor impairment and gait disturbance and interestingly they could not change their path during movement and their spontaneous exploratory activities were reduced (Figure 2.A). As an indicator of motor impairment, there was a significant reduction in total distance moved by VCR only treated animals compared with saline group (*P*<0.001). Lithium repaired the gait disturbance induced by VCR because total distance moved in VCR+Li 20 was significantly enhanced compared with single VCR treated group (*P*<0.01) and also there was no significant difference between VCR+Li 40 and saline group (*P*<0.05). No significant changes in total distance moved were determined in lithium only treated animals (*P*>0.05; Figure 2.B).

Nerve conduction velocity

There was a significant difference noticed between sciatic nerve conduction velocity recorded from saline and VCR group (*P*<0.001). Figure 3 shows that NCV was significantly increased in VCR and lithium treated animals (VCR+Li 20 group vs.VCR group: *P*<0.05; VCR+Li 40 group vs. saline *P*>0.05).

Histopathologic findings

Histopathologic examinations of longitude sections of sciatic nerve in VCR only treated rats showed a tangible deformation, swelling and degeneration of axon fibers (Figure 4), and also cross sections of respective nerves showed some marked pathological changes such as swelling in some nerve fibers, myelin sheet alteration, and slight demyelination, axonal shrinkage, axonal degeneration and total axon loss (Figure 5.A). Since fewer nerve fibers showed deformation and degeneration in VCR+Li treated groups, our study demonstrates that lithium was robustly able to reduce the pathological lesion related to VCR treatment (Figure 5.B and C).

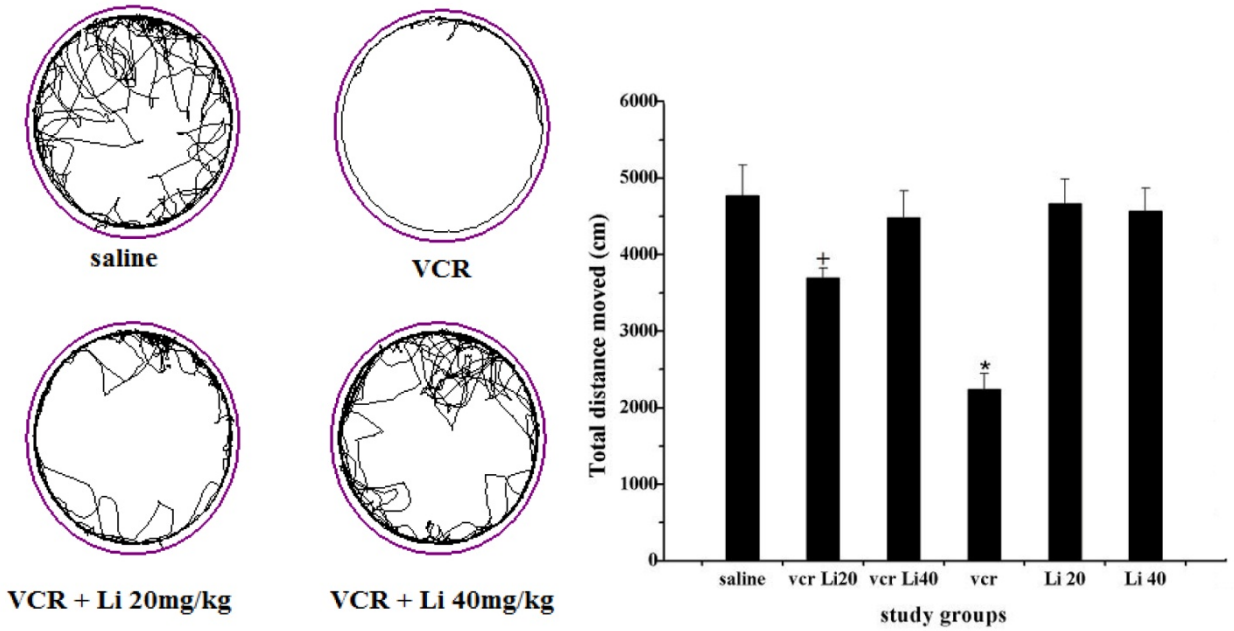

Figure 2. A Spontaneous exploratory activity as measured in an open field arena is altered in rats that suffering from neuropathy. It visually can be understood that VCR caused gait disturbance and lithium was able to ameliorate this disorder. The total distance moved within the open field arena (diameter 1.4 meter) was assessed over 10 min and it was significantly altered in VCR group compared with saline (*****P<0.001). In VCR+ Li 20 treated rats total distance moved was significantly higher than VCR only treated rats (**+**P<0.01) and there was no difference in total distance moved in VCR+ Li 40 versus saline (P>0.05).

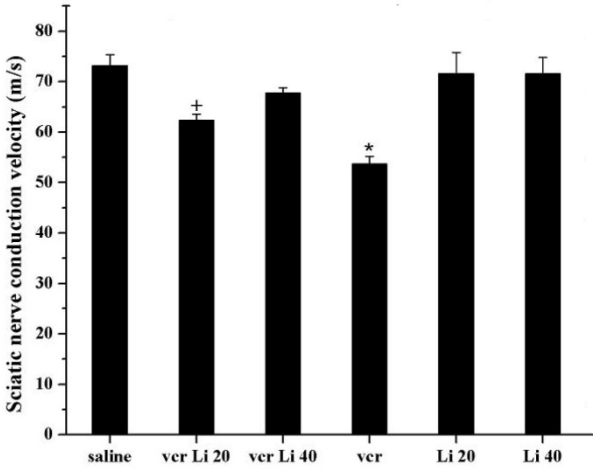

Figure 3. The sciatic nerve conduction velocity NCV value recorded from VCR treated and or lithium (20mg/kg or 40 mg/kg) treated rats. *P<0.001 vs. saline, +P<0.05 vs. VCR group

In addition as shown in table 1, there was a significant difference in G-ratio between VCR group and saline (P<0.001) while G-ratio was significantly increased in VCR+Li 20 group versus VCR group (P<0.05) and there was no significant diference between VCR+Li 40 group and saline (P<0.05). Lastly as shown in figure 6 prominent changes in DRG neurons were significantly evident in single VCR treated animals by increase in diameter of small cells and decrease in large cells. Moreover mean diameter of large cells in VCR group was significantly decreased versus saline group (P<0.001) while in VCR+Li 20mg/kg it was higher than only VCR treated group (P<0.01) and in VCR+Li 40 group it had no significant difference with saline (P>0.05). Mean diameter of small cells in VCR group was significantly increased versus saline (P<0.05) but there were no significant differences in diameter of small cells in VCR and lithium treated groups (P>0.05; Table 2).

Table 1. Morphometeric data on sciatic nerves from study groups, the results are reported as mean±S.EM. and n=15 for each group (3 section from each animal). *P<0.001 vs. saline, +P<0.05 vs. VCR group

Study groups	G ratio
Saline	0.42±0.02
VCR+ Li 20	0.34±0.01 +
VCR+ Li 40	0.38±0.02
VCR	0.29±0.02 *
Li 20	0.40±0.01
Li 40	0.41±0.01

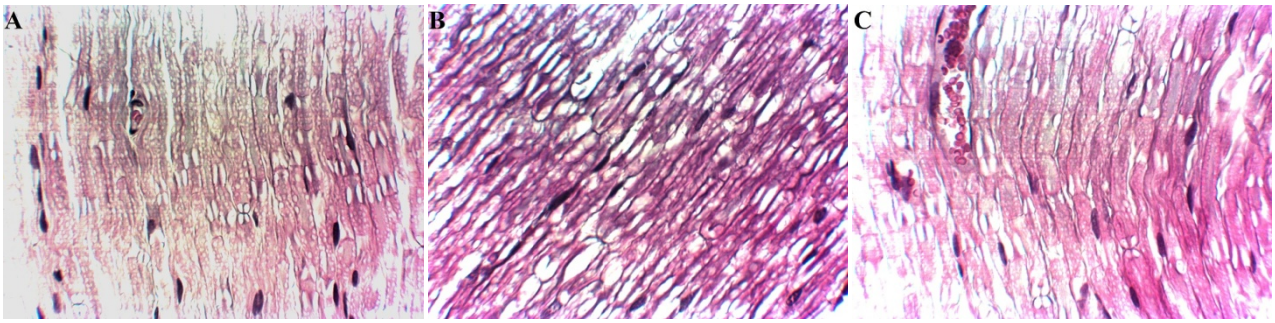

Figure 4. Longitudinal section of the sciatic nerves from saline (A), VCR treated group (B) and VCR+Li 40 group (C). A notable swelling and degeneration were observed in longitudinal section of the sciatic nerves in single VCR treated rats, but no marked differences were shown between VCR+Li and saline.

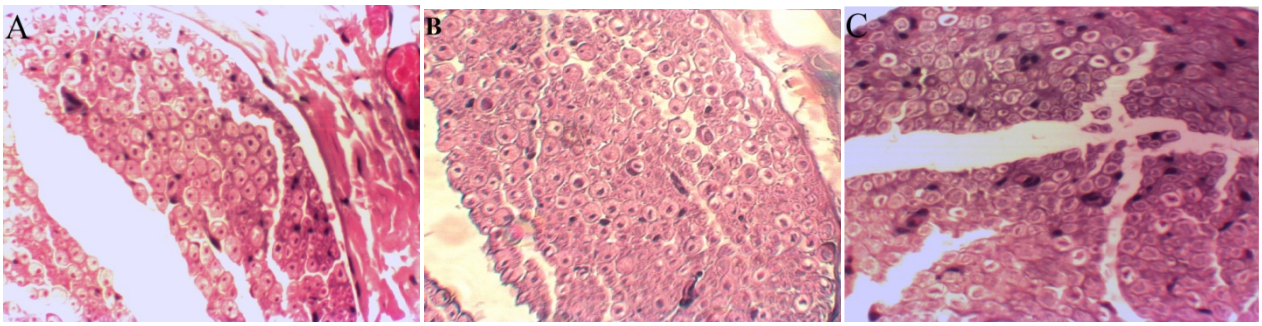

Figure 5. Sciatic cross sections of respective groups (shown in Figure 4), axonal swelling in some nerves and remarkable degeneration in other nerves and also demyelination were detected in VCR treated groups and lithium was able to repair these lesions induced by VCR as fewer changes were seen in VCR+ Li 20 and VCR+ Li 40 groups (H&E × 400).

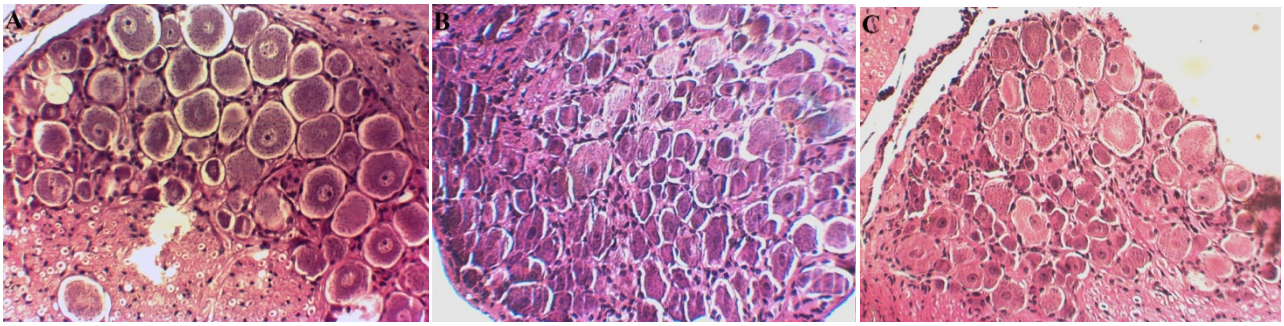

Figure 6. Dorsal root ganglion neurons of saline (A), VCR treated group (B) and VCR+Li 40 group (C), stained with H & E. In DRG of VCR treated rats (B) there were a marked reduction in number and diameter of A type cells (large cells) and also an increase in number and diameter of B type cells (small cells) while fewer changes were detected in VCR+Li 20 or 40. Original magnification (a_d) × 200.

Table 2. Morphometeric data on DRG neurons from all study groups are summarized in this table, the results are reported as mean ± S.E. and n=15 for each group (3 section from each animal). **P*<0.01 vs. saline, ***P*<0.001 vs. saline, +*P*<0.05 vs. VCR group.

Study groups	Large cell Diameter (µm)	Small cell Diameter (µm)
Saline	43.2±1.71	21.2±0.8
VCR+ Li 20	36.4±1.03 +	20.9±1.2
VCR+ Li 40	40.1±1.82	21.0±0.7
VCR	28.0±1.06 **	26.3±1.0 *
Li 20	39.1±1.20	22.5±1.2
Li 40	40.3±1.63	20.8±0.9

Discussion

Our finding shows that VCR administration at the dose of 0.3 mg/kg induced a marked peripheral sensorimotor neuropathy with behavioral, electrophysiological and histological alterations. PLC and ELC were measured at the 7th day of study (lithium pretreatment period) and at the day of sacrifice and we found that after about 7 days, lithium in plasma and erythrocytes had a steady state concentration and in normal housing situation they did not significantly change (14) and these concentrations were subtheraputic. Lithium at the dose of 20 mg/kg and 40 mg/kg was able to ameliorate the rate of mortality and general toxicity (8,14). Based on the results of electrophysiological and behavioral examinations we can safely say that lithium at doses of 20mg/kg and 40mg/kg significantly reduced sensorimotor neuropathy induced by VCR. There were recognizable differences in DRG and sciatic neurons between VCR and VCR + Li 20 mg/kg or 40mg/kg groups and these findings show that lithium repaired pathologic damages induced by VCR. Such as axonopathy, decrease in axon's diameter and demyelination. As conduction velocity could be a good indicator of axon's function, the NCV finding and histological analyses for study groups were parallel. What has emerged from this study showed the same results as Petrini *et al.* (9). Lithium has a wide variety of mechanisms of action; therefore it is difficult to suggest a possible mechanism for its neuroprotective property. Lithium plays an important role in microtubule dynamics and stability directly or indirectly via GSK3β (15-18). Bhattacharyya and Wolff reported that lithium in concentrations of 0.2–1.0 meq promotes tubulin polymerization and also showed that lithium protects microtubules against depolymerizing effects of colchicine or vinblastine (15). It has been widely accepted that lithium robustly protects some damaged cells against excitotoxicity via factors involved in cell survival pathway, including cAMP (cyclic adenosine monophosphate) response element binding protein, brain derived neurotrophic factor, and bcl2 (19, 20). Considering what has already been expressed, the possible mechanisms of interaction between lithium and VCR neuropathy might be due to its effect on factors involved in cell survival pathways as Balaei *et al*., reported the same results for the protective effect of lithium on cardiotoxicity induced by doxorubicine (14) or by its effect on microtubul stability.

In summary, the data presented here imply that lithium at the dose of 20 mg/kg and 40 mg/kg significantly ameliorates the sensorimotor neuropathy related to VCR. In order to employ this inexpensive, relatively safe neuroprotective drug in chemotherapeutic regimens that induce neuropathy, larger preclinical and clinical studies need to be performed.

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