

Effects of lithium chloride and methylprednisolone on experimental spinal cord injury

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ABSTRACT

Objectives. Antioxidant effects of lithium chloride (LiCl) and methylprednisolone were investigated in an experimental spinal cord injury. **Methods.** Spinal cord injury was performed by cerebral vascular clip with a closing force of 40 g; the duration of epidural compression was 30 seconds after T9-11 total laminectomy in the rat spine. The study was conducted in 4 groups. Group 1: sham (n=8), group 2: 0.9% saline (n=8), group 3: LiCl (n=8), group 4: methylprednisolone (n=8). Ketamine (60 mg/kg) and 2% xylazine (5 mg/kg) were used intraperitoneally as anesthesia protocol for the groups. The rats were sacrificed 24 hours after the injury and blood samples were taken. Total oxidant status (TOS), total antioxidant status (TAS), malondialdehyde (MDA) and tumor necrosis factor- α (TNF- α) level were analyzed. **Results.** Median (q1-q3) levels of TAS, TOS, MDA and TNF- α were statistically analyzed for the study groups. The TAS values of LiCl yielded statistically significant differences compared with group 1, 2 and 4 ($p<0.05$). The MDA values of LiCl and methylprednisolone groups were found to significantly differ between the sham and saline groups ($p<0.05$). There were no statistical differences between the study groups for the TNF- α and TOS values ($p>0.05$). **Conclusions.** LiCl seems to be an effective drug for experimental spinal cord injuries.

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Keywords: Spinal cord injury; experimental; treatment; lithium chloride; methylprednisolone

Introduction

Lithium chloride (LiCl) is used for the treatment of bipolar affective disorder [1, 2]. Treatment results of patients with spinal cord injury are still poor despite various treatment approach and efforts. The aim of the entire treatment effort is to prevent secondary tissue

damage [3-5]. There are reports suggesting that LiCl protects the cultured neurons against glutamate-induced excitotoxicity and apoptosis mediated by N-methyl-D-aspartate (NMDA) receptors [6]. It has also been reported that pretreatment with LiCl inhibits

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Ca²⁺ influx into the cultured cerebellar granule cells by approximately 50% [6]. This study aims to evaluate the antioxidant effect of LiCl on experimental spinal cord injury.

Methods

The ethical committee of the Osmangazi University School of Medicine, Eskisehir, Turkey, approved the study. Male and female adult Sprague Dawley rats (250–350 g) were randomly assigned to four experimental groups (n=8 each): Group 1 (sham), laminectomized but without spinal cord injury or treatment; Group 2 (saline), spinal cord injury with 0.9% saline treatment; Group 3 (LiCl), spinal cord injury with 50 mg/kg lithium chloride treatment; and, Group 4 (methyl prednisolone), spinal cord injury with 30 mg/kg methylprednisolone treatment. Spinal cord injury was performed following T₉–T₁₁ total laminectomy using a cerebral vascular clip, (closing force 40 g, epidural compression duration 30 seconds). Saline, lithium chloride or methylprednisolone was given intraperitoneally one hour after the trauma. Ketamine (60 mg/kg) and 2% xylazine (5 mg/kg) were administered intraperitoneally to induce anesthesia in all groups. The rats were euthanized 24 hours after spinal cord injury and blood samples were obtained for biochemical analysis. Total oxidant status (TOS), total antioxidant status (TAS), malondialdehyde (MDA) levels and tumor necrosis factor- α (TNF- α) levels were measured using commercially available assays. The TOS value was expressed in $\mu\text{mol H}_2\text{O}_2$ Eq./l, the TAS value was expressed in mmol Trolox Eq./l, and the TNF- α level value was expressed in pg/ml.

MDA levels were determined using the method of Ohkawa et al. [7]. Briefly, 0.5 ml plasma was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8%

thiobarbituric acid, and heated at 95 °C for 60 minutes. After cooling, 5 ml n-Butanol/Piridin (15:1 v/v) was added and the samples were centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and the absorbance at 532 nm was measured using a Shimadzu UV-1201 spectrophotometer (Shimadzu Corp, Japan). The MDA level was calculated using 1,1,3,3-tetraethoxy-propane as a standard and was expressed in nmol/ml.

Statistical Analysis

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Variables were expressed as median (25th percentiles-75th percentiles). Comparisons of continuous variables between the groups were performed using the Kruskal Wallis one-way analysis of variance and Dunn's Post Hoc test and using a Bonferroni t test with a corrected p value of 0.05/4. A two-sided $p < 0.05/4$ was considered statistically significant.

Results

We compared the effects of lithium chloride and methylprednisolone in an experimental spinal cord injury model. Multiple measures of oxidative or inflammatory status, including TOS, TAS, and the levels of MDA and TNF- α were studied. Median TOS values were not significantly different between the treatment groups (Table 1) ($p=0.463$). Median TAS values, however, were significantly reduced in the lithium-treated rats (Table 2) ($p < 0.001$). Both LiCl and methylprednisolone treatments lowered the median levels of MDA, a marker of lipid peroxidation, relative to the control and sham groups (Table 3) ($p < 0.001$). There were no statistically significant differences between the TNF- α levels of the treatment groups (Table 4) ($p=0.574$).

Table 1. TOS values ($\mu\text{mol H}_2\text{O}_2$ Eq./l) for the groups

Groups	TOS	Median (q1-q3)	<i>p</i>
Sham (n=8)	11.43	8.32-19.07	0.463
Control (n=8)	21.72	5.6-35.44	
Lithium (n=8)	8.23	5.26-13.32	
Methylprednisolone (n=8)	8.57	6.40-18.15	

TOS= total oxidant status

Table 2. TAS values (mmol Trolox-Equ./l) for the groups

Groups	TAS	Median (q1-q3)	<i>p</i>
Sham (n=8)	1.25	1.16-1.33	
Control (n=8)	1.39	1.20-1.58	<0.001 ^{a, b, c}
Lithium (n=8)	1.06	0.99-1.11	
Methylprednisolone (n=8)	1.23	1.13-1.37	

TAS=total antioxidant status, ^aThere is significant difference between Lithium and Methylprednisolone groups, ^bThere is significant difference between Lithium and Sham groups, ^cThere is significant difference between Lithium and Control groups

Table 3. MDA values (nmol/ml) for the groups

Groups	MDA	Median (q1-q3)	<i>p</i>
Sham (n=8)	1.50	1.40-1.65	
Control (n=8)	1.85	1.65-1.90	<0.001 ^{a, b}
Lithium (n=8)	1.00	0.85-1.16	
Methylprednisolone (n=8)	1.20	1.10-1.45	

MDA= malondialdehyde, ^aThere is significant difference between Lithium and Sham groups, ^bThere is significant difference between Lithium and Control groups

Table 4. TNF- α values (pg/ml) for the groups

Groups	TNF- α	Median (q1-q3)	<i>p</i>
Sham (n=8)	45.88	36.65-69.06	
Control (n=8)	54.19	26.79-75.41	0.574
Lithium (n=8)	30.81	25.99-62.79	
Methylprednisolone (n=8)	40.27	28.61-54.32	

(TNF- α)=tumor necrosis factor- α

Discussion

Methylprednisolone is used to treat a variety of neurological disorders involving white matter injury, including multiple sclerosis, acute disseminated encephalomyelitis, and spinal cord injury [8-11]. LiCl is used to treat to bipolar affective disorder, and schizophrenia [1, 2, 12].

Boku *et al.* [13] reported that LiCl and glucocorticoids affected adenosine diphosphate (ADP) proliferation, which is regulated by glycogen synthase kinase 3 beta (GSK-3 β) and β -catenin/T-cell factor (TCF) pathways. Young [14] reported that chronic administration of LiCl increased the levels of neurotropic factors in the brain. LiCl stimulates not only regeneration but also neurogenesis both in-vitro and in-vivo. LiCl causes new neurons to be produced in both injured and uninjured hippocampus. The mechanism appears to involve Wnt/ β -catenin signaling pathway. LiCl is also described as a potent neuroprotective agent [14]. Dill *et al.* [15] reported

that the administration of GSK-3 β inhibitors may facilitate the development of an effective treatment to white matter injuries including spinal cord trauma given the wide use of lithium in humans and that the inactivation of GSK-3 β promotes axonal growth and recovery in central nervous system.

Lee *et al.* [9] demonstrated in-vivo (spinal cord injury in rat), and in-vitro that methylprednisolone reversed AMPA-(alpha-amino-3-hydroxy-5-methylisoxazole-4 propionate) induced decreases in the expression of antiapoptotic Bcl-xL, caspase-3 activation, and DNA fragmentation in oligodendrocyte by the glucocorticoid receptor, and not by neurons. These protective effects were inhibited by the glucocorticoid receptor antagonists: mifepristone (RU486) and small interfering RNA (siRNA). Bailly Maitre *et al.* [16] reported that the same antiapoptotic effects were seen in human and rat hepatocyte cultures by dexamethasone. Methylprednisolone (30 mg/kg, iv) used in an in-vivo rat study. The spinal cords were

examined 24 hours after the spinal cord injury for molecular sign of apoptosis. Methylprednisolone was found selectively to attenuate oligodendrocyte cell death and demyelination [9].

Xu *et al.* [11] demonstrated that methylprednisolone selectively inhibits oligodendrocyte death via glucocorticoid receptor and upregulates the expression of B-cell lymphoma-extra large (Bcl-xL). They also found that signal transducer and activators of transcription 5 (STAT5) plays a key role in mediating the protection of oligodendrocytes by the methylprednisolone/glucocorticoid receptor signaling pathway. However, the subsequent molecular cascades underlying the upregulation of Bcl-xL remained unknown. It has been reported that methylprednisolone upregulates the expression of Bcl-xL via direct binding of the glucocorticoid receptor/STAT5 complex on the putative STAT5 binding site [11]. Antiapoptotic Bcl-xL is seated on the outer membrane of mitochondria, which include intrinsic apoptotic pathway, thus provide maintenance of membrane integrity [17]. Nescic-Taylor *et al.* [18] suggested that antiapoptotic Bcl-xL has an important role on adult neural cells, which promote neuronal survival. Cittelly *et al.* [19] reported that phosphorylation of Bcl-xL is a proapoptotic event in the neurons and also after spinal cord injury.

Mohn *et al.* [20] reported that NMDA receptors represent a subclass of glutamate receptors that play a critical role in neural development and physiology. NMDA receptor blockers cause behavioral alteration (schizophrenia) due to increased dopamine level such as phencyclidine intoxication mimicking schizophrenia. Phencyclidine is a noncompetitive antagonist of NMDA receptors. Nieollon *et al.* [21, 22] reported that glutamate and dopamine exhibit reciprocal actions at subcortical cell, therefore dopamine receptor blockade may act to balance glutamatergic insufficiency [20, 23, 24].

Nonaka *et al.* [6] reported that LiCl protects to the cultured neurons against glutamate-induced excitotoxicity and apoptosis mediated by NMDA receptors. Javitt [25] reported that NMDA receptor antagonists cause glutamatergic dysfunction, which causes schizophrenic symptoms. On the other hand, hyper-glutamatergic neurotoxicity can cause cognitive deficit in schizophrenia [26]. These reports support our findings, which showed that LiCl and methylprednisolone results in NMDA receptor blockade.

Our results suggest that LiCl causes glutamate

dysfunction via NMDA receptor blockade, which protects neuronal apoptosis. The MDA and TAS levels found in this paper support this hypothesis. Our result showed that lithium chloride had an antioxidant effect on the experimental rat spinal cord injury. The TAS level of the LiCl group was significantly higher than the other groups. MDA levels of the LiCl and methylprednisolone groups were statistically lower than the other groups. These results suggest that LiCl has a potent antioxidant activity as strong as methylprednisolone. Our results support the effecting mechanism of LiCl on glutamate-induced excitotoxicity and apoptosis mediated by NMDA receptors. Li inhibits Ca^{+2} influx into neural cells.

Conclusions

In conclusion, LiCl seems to be an effective drug as strong as methylprednisolone on experimental spinal cord injury.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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