Journal of Health Sciences

RESEARCH ARTICLE Open Access

Effects of individual or combined use of alpha-lipoic acid and methylprednisolone on malondialdehyde, superoxide dismutase, and catalase levels in acute spinal cord injury in rats

Alper Birgul[1], Murat Ugur[1], Gokhan Canaz[2]*, Mehmet Tokmak[3], Metin Kasap[4], Ibrahim Alatas[5], Huseyin Canaz[5]

[1]Department of Neurosurgery, Kilis State Hospital, Kilis, Turkey, [2]Department of Neurosurgery, Istanbul Bakirkoy Prof. Dr. Mazhar Osman Psychiatric Training and Research Hospital, Istanbul, Turkey, [3]Department of Neurosurgery, Medipol University, Istanbul, Turkey, [4]Department of Neurosurgery, Haseki Training and Research Hospital, Istanbul, Turkey, [5]Department of Neurosurgery, Sisli Florence Nightingale Hospital, Istanbul Bilim University, Istanbul, Turkey

ABSTRACT

Introduction: Recent studies have demonstrated that alpha-lipoic acid (ALA) has a strong antioxidant property and it exerts neurotrophic effects on the peripheral nerves. In this study, we investigated potential effects of ALA on secondary injury mechanisms as well as on apoptosis.

Methods: Forty Sprague-Dawley rats were equally divided into 5 groups, as follows: laminectomy (control), laminectomy + trauma (Trauma), laminectomy + posttraumatic methylprednisolone [MP] (Trauma + MP), laminectomy + posttraumatic ALA (Trauma + ALA), laminectomy + posttraumatic MP and ALA (Trauma + MP + ALA). Yasargil aneurysm clip method was used to induce the spinal cord injury. Twenty-four hours after the procedure the rats were sacrificed. Spinal cord samples were harvested to analyze malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels, as well as histopathological characteristics.

Results: The Kruskal-Wallis test (95% confidence level, $p < 0.05$) showed a statistically significant difference between the groups in MDA ($p = 0.006$), CAT ($p = 0.000$), and SOD ($p = 0.001$) levels. Pairwise comparisons, with Bonferroni correction, of control and Trauma group with the other groups, revealed a significant difference in CAT and SOD levels. Overall, our results showed that ALA administration significantly decreased MDA levels in Trauma + ALA compared to the same effect of MP in Trauma + MP group.

*Corresponding author: Gokhan Canaz,
Department of Neurosurgery, Istanbul Bakirkoy Prof. Dr. Mazhar Osman Psychiatric Training and Research Hospital,
Dr.Tevfik Sağlam Street No:25/2 Post Code: 34147 Bakirkoy/Istanbul. Phone: +90 212 409 15 15.
E-mail: gokhancanaz@gmail.com

Submitted: 10 November 2016/Accepted: 10 December 2016

DOI: https://doi.org/10.17532/jhsci.2016.409

UNIVERSITY OF SARAJEVO
FACULTY OF HEALTH STUDIES

Furthermore, ALA administration increased SOD and CAT levels in Trauma + ALA group. The combined use of ALA and MP demonstrated synergistic effects and yielded even more significant results.

Conclusions: A combined use of ALA and MP might provide a benefit in patients exposed to spinal cord injury. However, at present, further studies are required to confirm these results.

Keywords: Alpha-lipoic acid; methylprednisolone; malondialdehyde; superoxide dismutase; spinal cord ınjury

INTRODUCTION

A spinal cord injury (SCI) remains a challenging clinical entity due to high morbidity and mortality rates. Advancements in technology and dynamics of modern life have markedly increased the rate of SCI and related diseases. For example, among the major causes of SCI today are motor vehicle accidents, falls from height, acts of violence and industrial accidents frequently can be enumerated (1, 2). Furthermore, the number of traffic accidents and violent acts has been increasing in recent years and, specifically in Turkey, 1600-2000 severe cases of SCI are reported every year (3).

In SCI, the first trauma occurrs as a result of mechanical stroke and is known as a primary injury. Flexion, extension, dislocation, and penetrating injuries cause strain and rupture of neural elements or spinal cord vessels, which is in direct proportion to the severity of stroke. A pharmacological treatment that could prevent primary SCI is not developed yet. The prevention of injuries by ensuring the stabilization of the body using active and passive safety measures may decrease the severity of damage to some extent. Secondary cellular damage triggered by mechanical injury develops within hours after the primary injury, and is caused by metabolic and biochemical agents. Current studies have been focused on the cessation or slowing down the secondary injury cascade. The development of treatment strategies for SCI includes experimental models consisting of surgical, pharmacological, and physiological methods. In addition, pathophysiologic processes that are initiated after the injury have been explored and neuroprotective treatment options have been investigated (4). A number of chemical agents have been used to prevent existing neural tissue damage, including GM1 ganglioside, naloxone, thyrotropin-releasing hormon (TRH), nimodipine and tirilazad mesylate (5). Some of the agents decrease tissue damage, while others increase functional recovery. With regard to new neuroprotective agents, experimental studies on minocycline and erythropoietin have yielded promising results (5).

However, currently, only methylprednisolone (MP) has been reported as the accepted treatment for acute SCI (6, 7).

Alpha-lipoic acid (ALA) is a fatty acid with diverse functions and various mechanisms of action. More specifically, it is a cofactor in an enzyme complex which catalyzes decarboxylation of alpha-keto acids, such as pyruvate and alpha-ketoglutarate (8). Therefore, ALA is required for the regulation of glucose metabolism. In addition, ALA is a very potent antioxidant which functions together with the major antioxidants such as vitamin E, vitamin C, and glutathione [GSH] (8-15). Oxidative stress due to free oxygen radicals, and associated inflammatory response and apoptosis, play an important role in the process of secondary injury, which occurrs after an acute SCI (16). Cytokines that are released from the central nervous system cells (i.e., IL-1β, IL-6, and TNF-α) induce the process of apoptosis and inflammatory response (17). The two major pathways of apoptosis, namely receptor-dependent extrinsic and receptor-independent intrinsic pathways, are presumably activated in SCI (16). Receptor-dependent apoptosis pathway is stimulated by extracellular signals, especially by tumor necrosis factor (TNF). TNF rapidly accumulates in the injured areas following the onset of SCI. Signaling through Fas receptors in neurons, microglia, and oligo dendrocytes induces successive caspase activation. Among the activated caspases are caspase-8, caspase-3, and caspase-6. The activation of these caspases results in the death of the affected cells (17). In the alternative extrinsic apoptosis pathway, caspase-3 activates inducible nitric oxide synthase (iNOS) which results in programmed cell death (17). In the most recent *in vivo* and *in vitro* studies, strong antioxidant and metal-chelating properties of ALA have been demonstrated. Furthermore, ALA increased nerve growth factor (NGF) levels in peripheral nerves, and

consequently exerted its neurotrophic effects, while decreasing TNF-alpha levels (7-13).

Adjustment of dosage and duration of treatment, as well as dose-dependent side effects of ALA should be clarified, before its application in the management of SCI. In this study, we investigated the effects of ALA on the mechanisms of secondary injury and apoptosis pathways, as well as compared the anti-inflammatory and biochemical properties of ALA and ALA-MP in treating SCI.

METHODS

This study was performed at the Department of Experimental Animal Biology and Biomedical Application Techniques, Istanbul University. Forty adult Sprague-Dawley rats, produced in that laboratory and weighing between 280 and 300 g (median 283 g), were used. In our experiment, microsurgical instruments, normal size surgical instruments, and surgical microscope were used (D.F. Vasconcellos, Brasil) (Figure 1).

Groups

The rats were randomized equally into 5 groups:

1. Control - Normal spinal cord tissue specimens following laminectomy were used.
2. Trauma - Following laminectomy, an extradural injury was induced using Yasargil aneurysm clip (Aesculap, USA) under 0.7 N pressure, applied for 60 seconds.

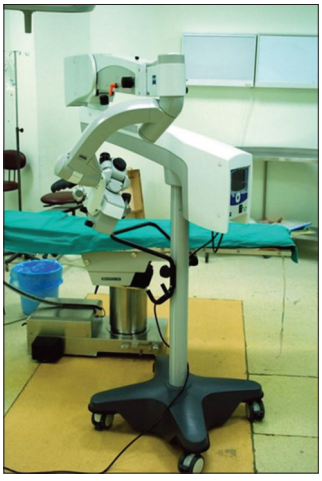

FIGURE 1. Surgical microscope.

3. Trauma + MP - Following laminectomy, an extradural injury was induced using Yasargil aneurysm clip under 0.7 N pressure, applied for 60 seconds. During the early postoperative period, intraperitoneal (i.p.) MP was given rapidly within 15 minutes at a dose of 30 mg/kg. After a 45-minute waiting period, MP was given at 4 equally divided i.p. doses of 5.4 mg/kg, within 23 hours.
4. Trauma + ALA - Following laminectomy, an extradural injury was induced using Yasargil aneurysm clip under 0.7 N pressure, applied for 60 seconds. During the early postoperative period, ALA was given via i.p. route at a dose of 50mg/kg.
5. Trauma + MP + ALA - Following laminectomy, an extradural injury was induced using Yasargil aneurysm clip under 0.7 N pressure, applied for 60 seconds. During the early postoperative period, i.p. MP was given rapidly within 15 minutes at a dose of 30 mg/kg together with i.p. ALA at a dose of 50 mg/kg.

Anesthesia

The rats subjected to surgery to induce the SCI were anesthetized using 60 mg/kg i.p. ketamine hydrochloride [HCL] (Ketalar, Parke Davis-Eczacıbaşı, Istanbul, Turkey) and 10 mg/kg i.p. xylazine (Rompun, Bayer, Istanbul, Turkey).

Surgical procedure

The rats subjected to the surgical procedure to induce SCI were anesthetized using i.p. ketamine HCL (60 mg/kg) and i.p. xylazine (10 mg/kg). The rats were stabilized in prone position, their thoracal regions were disinfected with povidone (PVD)-iodine, and shaved. Following the shaving, the surgical field was again disinfected with PVD-iodine. Under a surgical microscope, a 2-cm skin incision was made between T8 and T10 vertebrae. Then, through this skin incision, subcutaneous tissues were passed through, and paravertebral muscles were cleaved. Total laminectomy was perormed between T8 and T10, and the spinal cord was exposed (Figures 2 and 3). Next, Yasargil extradural aneurysm clip (FT217T) was applied for 60 seconds to the rats from Trauma, Trauma + MP, Trauma

+ ALA, and Trauma + MP + ALA group, under 0.7 N pressure, to induce the SCI. Following hemostasis, the layers were closed with 3/0 silk sutures (Figure 4).

Induction of SCI

In our study, following the total laminectomy performed between T8 and T10 vertebrae, as described by Tator and Rivlin (18), Yasargil extradural aneurysm clip was applied (FT217T) for 60 seconds to induce the SCI (19).

Pharmacological applications

ALA thioctacid solution for injection (Meda Pharma GmbH & Co. KG, Bad Homburg, Germany) was administered through i.p. route.

In the SCI-induced rats, the following procedures were applied:

Trauma + MP - During the early postoperative period, MP was delivered rapidly through i.p. route at a dose of 3.4 mg/kg within 15 minutes. After a waiting period of 45 minutes, MP was delivered at 4 equal doses of 5.6 mg/kg, within 23 hours.

Trauma + ALA - During the early postoperative period, ALA was delivered at a dose of 50 mg/kg through i.p. route.

Trauma + MP + ALA - During the early postoperative period, MP was delivered rapidly through i.p. route at a dose of 3.4 mg/kg, within 15 minutes. After a waiting period of 45 minutes, MP was delivered at 4 equal doses of 5.6 mg/kg within 23 hours together with 50 mg/kg ALA, via i.p. route.

At 24 hours postoperatively, rats were anesthetized with 60 mg/kg of i.p. ketamine HCL and 10 mg/kg of i.p. xylazine, and sacrificed using 100 mg/kg of i.p. thiopental sodium. Then, spinal cord specimens were harvested. Malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels were measured in these specimens.

Preparation of tissue homogenates

The spinal cord of the rats was weighed, mixed at a ratio of 1/9 with 0.1 M phosphate-buffered saline (PBS), and homogenized on ice at 10,000 rpm for one minute using a Miccrahomogenizer. The homogenized samples were centrifuged at 5000 rpm under + 4°C ambient temperature for 5 minutes, using a cooling centrifugal device.

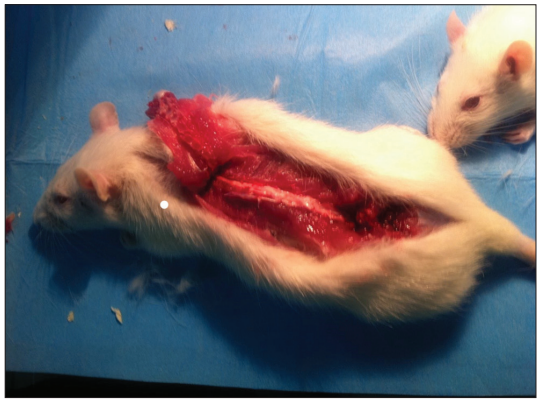

FIGURE 2. Macroscopic appearance of spinal cord before exposure to trauma.

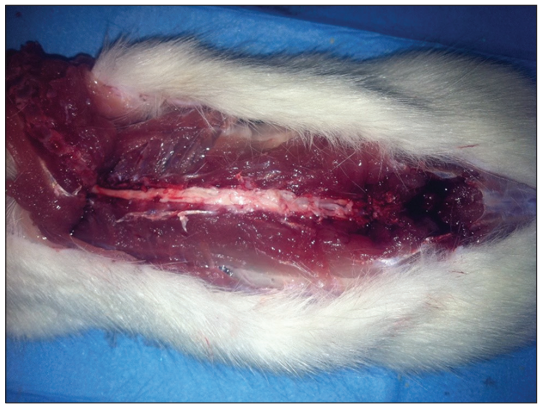

FIGURE 3. Macroscopic appearance of spinal cord before exposure to trauma.

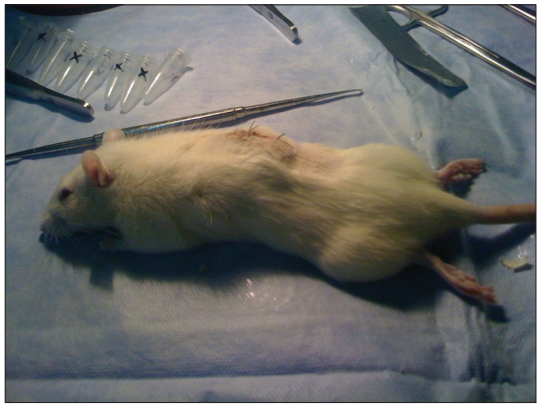

FIGURE 4. Post-operative.

The protein levels of supernatants of the homogenized samples were determined using a Siemens Advia immunoassay device (Siemens Healthcare Diagnostics Inc. USA). The tissue protein levels were determined using a CSF measurement kit (Thermo Fisher Scientific. USA). This method is based on the measurement of the changes in absorbance when a pyrogallol red-molybdate complex is bound to protonated basic amine groups of proteins, at acidic pH.

CAT levels were measured using spectrophotometric methods (Bioxytech catalase-520, OxisResearch Product kit - Oxis International. USA). A standard solution prepared by mixing the surfactant of PBS with catalase was placed on the specimen. Then, 10 mM of H_2O_2 contained in 500 µL PBS was added to this solution and incubated for one minute. After that, sodium azide was added. The prepared solution was placed in screw capped tubes and mixed without vortexing. A 20-µl aliquot of this mixture was pipetted into a tube, and 2 ml of horseradish peroxidase (HRP) in PBS and chromogenic mixture (110 ml chromogen in 110 µl HRP) was added. This mixture was again mixed without vortexing, and its absorbance was read at 520 nm after an incubation period of 10 minutes. All procedures were performed under room temperature. The results were expressed in U/g.

MDA levels were measured using spectrophotometric methods (Bioxytech MDA-5861 OxisResearch Product kit). Thiobarbituric acid reactive substances (TBARS) method is a nonspecific reaction which means that it reacts with both, free and protein-bound MDA. Therefore, we aimed to measure free MDA levels using the MDA-586 method after hydrolysis. In that way, other lipid peroxides, such as 4-hydroxy-alkenals, would not affect the results.

The method was based on the reaction between 2 moles of a chromogenic agent N-Methyl-2-phenylindole (NMPI) and MDA to produce a stable carbocyanine dye. The measurements were made using spectrophotometric methods and the results were read at 586 nm. Ten microlitres of probucol in methanol was pipetted into a tube and vortexed after the addition of 200 µl of homogenized tissue and 640 µl of diluted reagent-1 (NMPI). Afterwards, 150 µl of reagent-2 (concentrated HCl) was added and re-vortexed. The vortexed solution was incubated at 45 °C for 60 minutes, and centrifuged for 10 minutes at 10,000 rpm. The resultant clear supernatant was transferred into a spectrophotometer cuvette and read at 586 nm. The results were expressed as µmol/g of tissue.

SOD (EC 1.15.1.1.) activity was measured based on the reduction of nitroblue tetrazolium (NBT) by superoxide, which is produced by xanthine-xanthine oxidase system. The produced superoxide radicals reduce NBT, and the result is the formation of colored formazan dye. This complex gives a maximum absorbance at 560 nm (Cayman Chemical Company, Ann Arbor, USA). A colorimetric plate formed as the result of reactions was read at 560 nm using Biotek ELISA plate reader (BioTek. USA). Results were expressed as U/mg of wet tissue weight.

Statistical analysis

SPSS 22 (Statistical Package for Social Sciences) for Windows 10.0 (IBM – USA), was used for statistical analysis. Parameters with non-normal distribution were compared between the groups using the Kruskal–Wallis H test, and for the determination of the group which differed, Mann Whitney U test was used. Intragroup comparisons were performed using the Wilcoxon signed-rank test. The results were evaluated at a significance level of $p < 0.05$, and within 95 % confidence interval (CI). Mann –Whitney test with Bonferonni correction was performed between both the control, and the trauma groups versus other groups. The control group was compared with other groups as pairs as follows: (Groups 1, and 2), (Groups 1, and 3), (Groups 1, and 4), (Groups 1, and 5) at an accepted significance level of $p < 0.0125$. Trauma group was compared with the pairs of other groups, as follows: Trauma versus Trauma + MP, Trauma versus Trauma + ALA, and Trauma versus Trauma + MP + ALA groups, with a significance level set at $p < 0.0166$.

RESULTS

The results of the Kruskal–Wallis H test showed a statistically significant intergroup difference in MDA ($p = 0.006$), CAT ($p = 0.000$), and SOD levels [$p = 0.001$] (Tables 1 and 2, Figures 5, 6, and 7).

The results of the Mann–Whitney test (with a Bonferonni correction) did not show a significant

TABLE 1. Data set. MDA, CAT and SOD levels of groups

GROUP	MDA (µmol/mg protein)	CAT (U/mg protein)	SOD (mU/mg protein)
Control	6.3	6.9764	14.76
	4.4	8.1392	14.26
	5.8	7.692	22.96
	4.56	8.7656	13.26
	6.53	14.9396	18.46
	6.52	16.8184	17.82
	4.76	14.4028	21.96
	6.4	9.6604	23.66
Trauma	12.61	3.49344	8.1
	10.97	1.809	4.02
	8.92	3.04806	3.46
	9.53	3.16025	9.33
	12.2	3.01056	5.98
	11.84	2.9689	8.97
	16.86	1.0424	7.98
	11.84	1.29474	5.985
Trauma+MP	4.4	4.34344	10.125
	11.2	4.66205	3.312
	5	4.45085	4.446
	11.2	3.36909	9.25
	7.4	5.74728	10.55
	11.2	2.18882	12.238
	6.1	4.02145	9.35
	13.02	4.26676	5.05
Trauma+ALA	13.43	5.376	12.4
	9.74	5.18141	14.8
	13.23	10.2271	13.15
	8.92	6.88128	15.6
	3.38	6.90854	12.8
	5.84	7.8007	12.8
	2.23	7.51149	11.2
	6.92	6.93523	15.35
Trauma+ALA+MP	8.32	6.09274	19.14
	5.63	5.99726	13.84
	6.1	10.1481	8.16
	8.51	17.5106	16.75
	6.45	3.25107	5.19
	5.84	7.76844	15.57
	5.63	4.32382	16
	7.51	14.9932	18.42

ALA: alpha-lipoic acid; MP: methylprednisolone; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase

TABLE 2. MDA, CAT and SOD levels of groups. Data are given as mean±standard deviation

Study Groups	MDA µmol/mg protein	CAT U/mg protein	SOD mU/mg protein
Group 1-Control	5.65±0.93	10.92±3.83	18.39±4.11
Group 2-Trauma	11.84±2.40	2.47±0.94	6.72±2.21
Group 3-MP	8.69±3.33	4.13±1.02	8.04±3.28
Group 4-ALA	7.96±4.16	7.10±1.56	13.51±1.56
Group 5-ALA+MP	6.74±1.19	8.76±5.11	14.13±4.95

ALA: alpha-lipoic acid; MP: methylprednisolone; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase

difference in MDA levels between the control and treatment groups (Trauma + MP, Trauma + ALA, and Trauma + MP + ALA).

With regard to the CAT levels, a significant difference was not detected only between the control and Trauma + ALA + MP group (p = 0.249). Regarding the SOD activity, a significant difference was not detected only between the control and Trauma + ALA and Trauma + MP + ALA groups (p = 0.016 and p = 0.172, respectively).

A significant difference was detected between Trauma and Trauma + MP + ALA group in MDA levels (p = 0.001). Trauma group was significantly different from the other three groups with respect to CAT levels. As for the SOD levels, a significant difference was detected between Trauma group and Trauma + ALA and Trauma + MP + ALA groups (p = 0.001 and p = 0.012, respectively). The mean SOD level was relatively higher in Trauma + MP + ALA group.

Multiple group comparisons

Since the level of MDA did not differ significantly between the groups (p = 0.085), except between the control and Trauma group (p = 0.001) (Table 3).

DISCUSSION

The protection from secondary injury in cases with acute SCI is called neuroprotection. Numerous medical approaches, including surgeries, drug treatments, regulation of tissue oxygenization, spinal decompression, and stabilization of the spine have

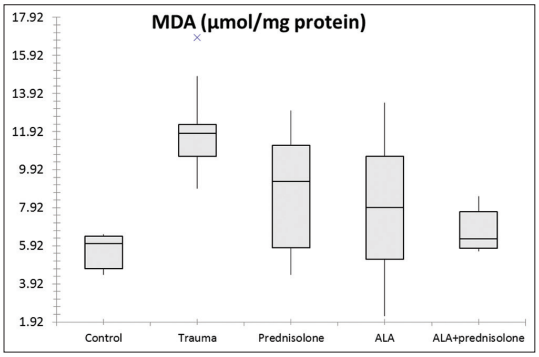

FIGURE 5. Malondialdehyde (MDA) values in experimental and control groups.

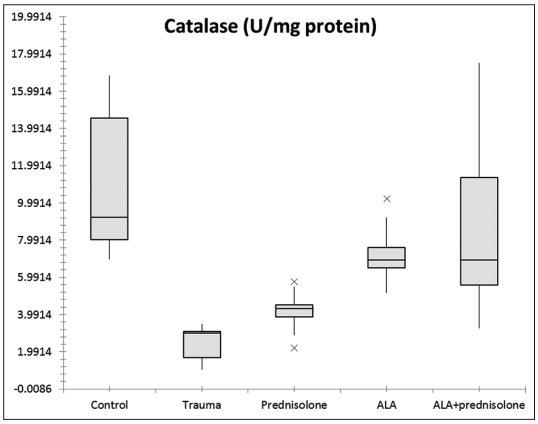

FIGURE 6. Catalase (CAT) values in experimental and control groups.

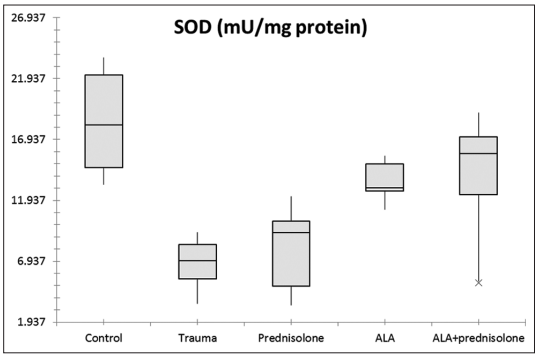

FIGURE 7. Superoxide dismutase (SOD) values in experimental and control groups.

been used. Although, for the last 20 years, a number of studies investigating SCI have been performed, a standardized treatment that could be used in humans has not been established yet.

TABLE 3. Results of Kruskal–Wallis H test (95% CI and $p < 0.05$)

	MDA	CAT	SOD
Chi-Square	8.199	31.070	17.744
df	4	4	4
Asymp Sig	0.085	0.000	0.001

MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; Asymp Sig: Asymptotic significance

Secondary injury starts within minutes or hours following the primary injury, and persists for weeks. Because it is not possible to treat primary injury, the rationale of investigating secondary injuries is to discover pharmacologic agents that will preserve the vitality of tissues, their connections with distal neurons, protect and increase the "endurance" of neurons in the injured area, or halt the pathological processes (20).

Dramatic pathological changes in the spinal cord lesion within first few days after the injury, represent the most important subject of clinical and experimental observations (13). The concept of dual mechanism in the spinal cord injury was introduced at the beginning of 1900s by Allen who demonstrated that progressive damage occurrs in patients with spinal cord injuries (21). Following SCI, a series of pathological changes occurs in the spinal cord, including bleeding, edema, demyelinization, axonal and neuronal necrosis, and cavity. Ducker demonstrated gradual worsening of these pathological changes up to 6 days after the injury (22). Nemecek defined this necrotic process as autodestruction (23). Attempts to prevent the secondary injury in SCI target glutamatergic, cholinergic and catecholaminergic neurotransmission systems, production of free radicals, lipid peroxidation, calcium and other anion channels, growth factors, inflammatory processes, endogenous opioid receptors, enzymes, apoptotic cell death, and regeneration mechanisms.

The cell death occurring after SCI has been explained by necrosis, developed as a consequence of ischemic and inflammatory reactions associated with tissue damage (24). In addition, the important role of apoptosis (previously thought to be present only during the development of central nervous system) and neuronal damage in posttraumatic spinal cord degeneration has already been recognized (25, 26).

Crowe et al. reported that the mechanisms of secondary injury, triggered following a primary SCI, induce apoptosis and progressive neurodegeneration (27). Anderson et al. demonstrated that neurological damage emerging after an acute SCI is related to necrosis which occurs after the primary and subsequent secondary injury; furthermore, apoptosis is observed at a later stage (28). Liu et al. revealed that neuronal and glial apoptosis occurs within 4 and 9 days after SCI (28). According to Lu et al., neuronal and glial apoptotic cells were not observed within the first 5 minutes after a contusion injury; nevertheless, the apoptotic process was detected in the injured area between the first 4 hours and 14 days after the injury, and it peaked at the 24th hour (29). It has also been showed that in SCI, the activation of caspase-1 and caspase-3 induces apoptosis (30). In the study by Emery et al., the caspase-3 activation was demonstrated in the human spinal cord, following SCI (29). Li et al. emphasized that the inhibition of caspase-3 after an induced SCI in rats, markedly decreased the injured area and improved the motor functions (31). Recently, therapeutic prophylaxis targeted at the apoptotic cascade in cells, after SCI, has been in focus (32).

In this study, we investigated the neuroprotective effects of ALA alone or in combination with MP on MDA, SOD, and CAT levels in SCI-induced rats.

Lipid peroxidation levels can be determined via the intermediate products produced during lipid peroxidation, such as MDA (33). In the body, different antioxidant mechanisms prevent the production of free radicals or eliminate existing free radicals (34). In cells, polyunsaturated fatty acids in the membrane phospholipids can be easily oxidized spontaneously or by binding to oxidative metabolites, and transformed to peroxide derivatives. The most potent antioxidant is vitamin E, which prevents this cascade accompanied by the production of free oxygen radicals at the level of cell membrane, and neutralizes the existing free oxygen radicals. Other antioxidant systems in the body (vitamin C, glutathione peroxidase, beta-carotene, etc.) are not as effective as vitamin E in this pathway. However, in general, due to beneficial effects on the membrane lipids as well as on subcellular structures, vitamins protect the membrane against oxidative damage (35).

In this study, we biochemically investigated the neuroprotective effects of ALA on SOD, CAT, and MDA levels.

Barut et al. measured lipid peroxidation and MDA levels during the early period after SCI (at posttraumatic 1 and 15 minutes, and 1, 2 and 4 hours), and detected that MDA levels increased starting from 15 minutes after the injury, then peaked at 1 hour, and decreased afterwards (33).

Kaynar et al. evaluated the efficacy of nimodipine and N-acetyl cysteine (NAC) by measuring tissue lipid peroxidation levels, one hour after an induced SCI. Based on the MDA measurements, a single dose of NAC had no effect on the peroxidation of lipid membranes in the experimentally induced SCI (36).

Kaptanoglu et al. investigated the antioxidant effects of thiopental and propofol in experimental cases with SCI, as well as the microstructural effects of the SCI. They demonstrated increased levels of MDA, the indicator of lipid peroxidation, in rats subjected to cerebral contusion. They also demonstrated that thiopental and propofol decreased lipid peroxidation; however, propofol could not reverse the microstructural pathological changes (37).

In our study, the MDA levels were as follows: control (5.65 ± 0.93 μmol/mg protein), Trauma (11.84 ± 2.40 μmol/mg protein), Trauma + MP (8.69 ± 3.33 μmol/mg protein), Trauma + ALA (7.96 ± 4.16 μmol/mg protein), and Trauma + MP + ALA (6.74 ± 1.19 μmol/mg protein). The MDA levels did not differ significantly between the groups ($p = 0.085$). However, in the intergroup comparisons, a statistically significant difference was observed in MDA levels between the control and Trauma group ($p = 0.001$).

Hanci et al. compared the efficacy of MP, NAC, and prednisolone + NAC treatment in an experimental SCI. The mean MDA levels were significantly lower in the MP, NAC, and MP + NAC groups when compared with the control group ($p < 0.05$). However, the mean SOD levels were significantly higher in MP, NAC, and MP + NAC groups compared to their control group ($p < 0.05$). On contrary, a significant difference was not observed between MP and NAC group in the mean MDA and SOD values (38).

CONCLUSION

In our study, the administration of ALA alone decreased significantly the MDA levels compared to the effect of MP and, at the same time, ALA increased the SOD and CAT levels. A combined use of ALA and MP demonstrated synergistic effects, and yielded even more significant results. In our opinion, the combined use of ALA and MP could provide the benefit for patients with SCI. However, additional studies are required to confirm our results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Tator CH, Carson JD, Edmonds VE. Spinal injuries in ice hockey. Clinics in sports medicine. 1998 Jan;17(1):183-94. PubMed PMID: 9475982.
2. Torg JS, Naranja RJ, Jr., Pavlov H, Galinat BJ, Warren R, Stine RA. The relationship of developmental narrowing of the cervical spinal canal to reversible and irreversible injury of the cervical spinal cord in football players. The Journal of bone and joint surgery American volume. 1996 Sep;78(9):1308-14. PubMed PMID: 8816644.
3. Karamehmetoglu SS, Unal S, Karacan I, Yilmaz H, Togay HS, Ertekin M, et al. Traumatic spinal cord injuries in Istanbul, Turkey. An epidemiological study. Paraplegia. 1995 Aug;33(8):469-71. PubMed PMID: 7478742.
4. Kwon BK, Oxland TR, Tetzlaff W. Animal models used in spinal cord regeneration research. Spine. 2002 Jul 15;27(14):1504-10. PubMed PMID: 12131708.
5. Kwon BK, Fisher CG, Dvorak MF, Tetzlaff W. Strategies to promote neural repair and regeneration after spinal cord injury. Spine. 2005 Sep 1;30(17 Suppl):S3-13. PubMed PMID: 16138063.
6. Hugenholtz H, Cass DE, Dvorak MF, Fewer DH, Fox RJ, Izukawa DM, et al. High-dose methylprednisolone for acute closed spinal cord injury--only a treatment option. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques. 2002 Aug;29(3):227-35. PubMed PMID: 12195611.
7. [no_author_listed]. Pharmacological therapy after acute cervical spinal cord injury. Neurosurgery. 2002 Mar;50(3 Suppl):S63-72. PubMed PMID: 12431289.
8. Takaoka M, Kobayashi Y, Yuba M, Ohkita M, Matsumura Y. Effects of alpha-lipoic acid on deoxycorticosterone acetate-salt-induced hypertension in rats. European journal of pharmacology. 2001 Jul 20;424(2):121-9. PubMed PMID: 11476758.
9. Cameron NE, Jack AM, Cotter MA. Effect of alpha-lipoic acid on vascular responses and nociception in diabetic rats. Free radical biology & medicine. 2001 Jul 1;31(1):125-35. PubMed PMID: 11425498.
10. Keegan A, Cotter MA, Cameron NE. Effects of diabetes and treatment with the antioxidant alpha-lipoic acid on endothelial and neurogenic responses of corpus cavernosum in rats. Diabetologia. 1999 Mar;42(3):343-50. PubMed PMID: 10096788.
11. Somani SM, Husain K, Whitworth C, Trammell GL, Malafa M, Rybak LP. Dose-dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. Pharmacology & toxicology. 2000 May;86(5):234-41. PubMed PMID: 10862506.
12. Garrett NE, Malcangio M, Dewhurst M, Tomlinson DR. alpha-Lipoic acid corrects neuropeptide deficits in diabetic rats via induction of trophic support. Neuroscience letters. 1997 Feb 7;222(3):191-4. PubMed PMID: 9148247.
13. Sehirli O, Sener E, Cetinel S, Yuksel M, Gedik N, Sener G. Alpha-lipoic acid protects against renal ischaemia-reperfusion injury in rats. Clinical and experimental pharmacology & physiology. 2008 Mar;35(3):249-55. PubMed PMID: 17941895.
14. Genovese T, Mazzon E, Esposito E, Muia C, Di Paola R, Di Bella P, et al. Role of endogenous glutathione in the secondary damage in experimental spinal cord injury in mice. Neuroscience letters. 2007 Aug 9;423(1):41-6. PubMed PMID: 17669594.
15. Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Lund DD, Yorek MA. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. Diabetes. 2001 Aug;50(8):1927-37. PubMed PMID: 11473057.
16. Guzel A. Pathophysiology and Physiology of Spinal Injury. Cerrehpasa University Medicine Faculty Journal. 2006;28:73-8.
17. Wei Y, Fan T, Yu M. Inhibitor of apoptosis proteins and apoptosis. Acta biochimica et biophysica Sinica. 2008 Apr;40(4):278-88. PubMed PMID: 18401525.
18. Rivlin AS, Tator CH. Regional spinal cord blood flow in rats after severe cord trauma. Journal of neurosurgery. 1978 Dec;49(6):844-53. PubMed PMID: 731301.
19. Faden AI. Therapeutic approaches to spinal cord injury. Advances in neurology. 1997;72:377-86. PubMed PMID: 8993713.
20. von Euler M, Li-Li M, Whittemore S, Seiger A, Sundstrom E. No protective effect of the NMDA antagonist memantine in experimental spinal cord injuries. Journal of neurotrauma. 1997 Jan;14(1):53-61. PubMed PMID: 9048311.
21. Suzer T, Coskun E, Islekel H, Tahta K. Neuroprotective effect of magnesium on lipid peroxidation and axonal function after experimental spinal cord injury. Spinal cord. 1999 Jul;37(7):480-4. PubMed PMID: 10438114.
22. Ducker TB, Kindt GW, Kempf LG. Pathological findings in acute experimental spinal cord trauma. Journal of neurosurgery. 1971 Dec;35(6):700-8. PubMed PMID: 5000662.
23. Nemecek S. Morphological evidence of microcirculatory disturbances in experimental spinal cord trauma. Advances in neurology. 1978;20:395-405. PubMed PMID: 676903.
24. Wrathall JR, Teng YD, Marriott R. Delayed antagonism of AMPA/kainate receptors reduces long-term functional deficits resulting from spinal cord trauma. Experimental neurology. 1997 Jun;145(2 Pt 1):565-73. PubMed PMID: 9217092.
25. Bellamy CO, Malcomson RD, Harrison DJ, Wyllie AH. Cell death in health and disease: The biology and regulation of apoptosis. Seminars in cancer biology. 1995 Feb;6(1):3-16. PubMed PMID: 7548839.
26. Zileli M. Omurilik ve omurilik cerrahisi. Izmir: Meta Basim Matbaacilik; 2002.
27. Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS. Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nature medicine. 1997 Jan;3(1):73-6. PubMed PMID: 8986744.
28. Anderson DK, Means ED, Waters TR, Green ES. Microvascular perfusion and metabolism in injured spinal cord after methylprednisolone treatment. Journal of neurosurgery. 1982 Jan;56(1):106-13. PubMed PMID: 7054403.
29. Lu J, Ashwell KW, Waite P. Advances in secondary spinal cord injury: role of apoptosis. Spine. 2000 Jul 15;25(14):1859-66. PubMed PMID: 10888960.
30. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. Journal of neurotrauma. 1995 Feb;12(1):1-21. PubMed PMID: 7783230.
31. Li M, Ona VO, Chen M, Kaul M, Tenneti L, Zhang X, et al. Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse

model of traumatic spinal cord injury. Neuroscience. 2000;99(2):333-42. PubMed PMID: 10938439.

32. Asadullah K, Woiciechowsky C, Docke WD, Liebenthal C, Wauer H, Kox W, et al. Immunodepression following neurosurgical procedures. Critical care medicine. 1995 Dec;23(12):1976-83. PubMed PMID: 7497720.
33. Barut S, Canbolat A, Bilge T, Aydin Y, C.Cokneşeli B, Kaya U. Lipid peroxidation in experimental spinal cord injury: Time-level relationship. Neurosurg Rev. 1993 1993/03/01;16(1):53-9. English.
34. Agrawal SK, Nashmi R, Fehlings MG. Role of L- and N-type calcium channels in the pathophysiology of traumatic spinal cord white matter injury. Neuroscience. 2000;99(1):179-88. PubMed PMID: 10924962.
35. Schwab ME, Bartholdi D. Degeneration and regeneration of axons in the lesioned spinal cord. Physiological reviews. 1996 Apr;76(2):319-70. PubMed PMID: 8618960.
36. Kaynar MY, Erdincler P, Tadayyon E, Belce A, Gumustas K, Ciplak N. Effect of nimodipine and N-acetylcysteine on lipid peroxidation after experimental spinal cord injury. Neurosurg Rev. 1998;21(4):260-4. PubMed PMID: 10068187.
37. Kaptanoglu E, Sen S, Beskonakli E, Surucu HS, Tuncel M, Kilinc K, et al. Antioxidant actions and early ultrastructural findings of thiopental and propofol in experimental spinal cord injury. Journal of neurosurgical anesthesiology. 2002 Apr;14(2):114-22. PubMed PMID: 11907391.
38. Hanci V, Kerimoglu A, Koca K, Baskesen A, Kilic K, Tastekin D. The biochemical effectiveness of N-acetylcysteine in experimental spinal cord injury in rats. Ulusal travma ve acil cerrahi dergisi = Turkish journal of trauma & emergency surgery: TJTES. 2010 Jan;16(1):15-21. PubMed PMID: 20209390.