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Trace element, antioxidant and oxidant levels in spinal cord injury: different perspective on the effects of valproic acid

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Abstract. – OBJECTIVE: One of the most pronounced phenomena of spinal cord injury (SCI) pathology is various changes caused by oxidative stress due to secondary damage. In recent years, it has been understood that valproic acid (VPA) has neuroprotective properties other than its clinical effect. The aim of this study is to investigate whether there is a change in antioxidant activity and trace the element level due to SCI-induced secondary damage, and to examine whether VPA has an effect on this change.

MATERIALS AND METHODS: Experimentally, spinal damage was induced in a total of sixteen rats by compressing the infrarenal and iliac bifurcation parts of the aorta for 45 minutes and these rats were equally divided into SCI (control) and SCI + VPA groups. The treatment group was injected with VPA (300 mg/kg) intraperitoneally once following SCI. In addition, the motor neurological functions of both groups after SCI were evaluated with the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale and Rivlin's angle of incline test. The spinal cord tissues of both groups were homogenized and the supernatants were separated for biochemical analysis.

RESULTS: The results showed that SCI significantly reduced catalase (CAT), glutathione peroxidase (GPx), total antioxidant status (TAS), magnesium (Mg), zinc (Zn) and selenium (Se) levels and increased total oxidative status (TOS), oxidative stress indices (OSI), chromium (Cr), iron (Fe), and copper (Cu) in damaged spinal cord tissue. In particular, the administration of VPA prior to the significant increase in the effect of SCI-secondary damage turned these negative findings into positive.

CONCLUSIONS: Our findings show that the spinal cord tissue damaged during SCI is protected against oxidative damage thanks to the neuroprotective property of VPA. Furthermore, it is an important finding that this neuroprotective mechanism contributes to the maintenance of the level of essential elements and antioxidant activity against SCI-induced secondary damage.

Key Words:
Spinal cord injury, Trace elements, Valproic acid, Oxidative stress, Antioxidant activity.

Introduction

Spinal cord injury (SCI) is a life-threatening event that results in multiple organ damage and sometimes permanent physical deformities for both humans and animals. The mechanisms underlying organ damage are not fully known. However, local complications that usually begin with primary damage, followed by the secondary damage with a series of biochemical reactions, are thought to be the underlying reasons[1]. At the beginning of biochemical changes in secondary damage prone to treatment, ion imbalance is followed by oxidative stress and inflammation. When left untreated, ionic imbalance plays a very important role in reactions leading to the death of spinal cord cells[2]. Valproic acid (VPA) shows that it has potentially anti-oxidation and anti-inflammatory properties and prevents complications such as oxidative stress after ischemia/reperfusion[3]. When we examine all the studies, we can say that the studies on the effect of VPA on ion imbalance after SCI are quite limited. However, there are studies in which there are various mechanisms underlying the prevention of oxidative stress with the application of VPA[4]. For example, inhibition of VPA-mediated histone deacetylases (HDACs) is a protective effect against motoneuronal death in amyotrophic lateral sclerosis, which develops as a result of oxidative stress. DNA-histone relationships are very important both in terms of chromatin structure and gene regulation. The inhibition of HDACs by VPA leads to his-

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tone acetylation. This condition can increase the relaxation of the tightly wound DNA structure and the access of transcription factors, thereby leading to the realization of gene transcription[5]. In addition, neurogenesis activation by VPA treatment can create a highly protected microenvironment in the neuronal structure as a result of the expression of the growth factor brain-derived neurotrophic factor (BDNF) in young neurons[6]. A recent study[7] reported that VPA and retinoic acid treatment combinations are compatible, and that retinoic acid promotes neurogenesis of spinal cord ependymal cells (SCECs, plays an important role in SCI recovery) and reduces astrocytic differentiation. In this way, VPA has been shown[8] to have neuroprotection and neurogenesis effects in brain damage and ischemia/reperfusion injuries. This results in the development of healthy proliferating neurons and motor abilities.

SCI, caused by factors including temporary vascular clamping, is exposed to the effects of ischemia due to such reasons as low blood flow and limited anaerobic metabolic capacity. Reperfusion-induced reactive oxygen species (ROS) following ischemia can lead to the triggering of oxidative stress, numerous oxidant mechanisms, inflammation and an increase in the apoptotic mechanism, and further tissue damage[9]. Recent research[10] has focused on the sequence of occurrence of biochemical changes, such as ion imbalance, oxidative damage and inflammation, leading to brain cell death due to brain ischemia. Therefore, ion imbalance plays an active role in the initiation of ischemic events such as cerebral ischemia and the formation of other oxidant mechanisms. SCI-induced ischemia/reperfusion stimulates the production of ROS and reactive nitrogen species (RNS). The increase of these types of reagents causes damage to elements of the cell membrane such as lipids, proteins, and nucleotides (DNA). The reaction of ROS with long-chain unsaturated fatty acids brings about the further spread of oxidative damage and disruption of membrane permeability, which eventually result in cell death[11]. The characteristics of the structure of the spinal cord, such as its high composition of polyunsaturated fatty acids and its limited antioxidant capacity, make it very vulnerable to oxidative stress after injury[12]. According to the mentioned findings, agents with neuroprotective and neurogenic effects, such as VPA, are promising in the treatment of such injuries due to the ability to minimize the effects of secondary damage after SCI. The body can limit normal ROS production, but it has developed a complex antioxidant system to limit the production that occurs beyond this limit for various reasons (such as SCI).

One of the most important mechanisms of this system is antioxidant enzymes, while the other is non-enzymatic antioxidants. Enzymatic antioxidants in the body protect organs such as the spinal cord and brain that are prone to oxidative damage that CAT, being one of the strongest members of the defense barrier, has the function of catalyzing the reduction of hydrogen peroxide radicals, thus reducing the tendency of brain and spinal cord tissues to oxidative damage. Similarly, GPx is an enzyme that is responsible for the breakdown of hydrogen peroxide, harmful to the mitochondria, down to water, and is important and essential in the protection of the cell. It is known[14] that both CAT and GPx activity is stable in the defense of ROS attacks. On the contrary, the reduced activities of these two antioxidants have been reported[15] to damage the brain and spinal cord tissue.

The authors reported[15] that it is very important to maintain the levels of several trace elements necessary both for the spinal cord to preserve normal functions and for SCI. In addition, these elements have an important role in the functioning of enzymatic antioxidants, which prevent oxidative damage that allows the spread of secondary damage developing after SCI. For example, Se, which is necessary for the function of GPx, prevents the oxidation of the cell bodies of neurons thanks to its antioxidant properties, thus allowing it to remain healthy. Cu and Zn, which have antioxidant properties such as Se, are important metalloenzymes found in living organisms[16]. They are present in the structure of CAT, one of the antioxidant enzymes, and thanks to their anti-inflammatory and antioxidant properties, they protect organs containing long-chain unsaturated lipids from oxidation. Also, the element Cu at normal levels is present in the biologically active part of superoxide dismutase (SOD)[17]. However, an excessive increase in Cu triggers oxidative stress and thus serves as a significant function in the etiology of various neurodegenerative disorders[18]. The human body needs the element Cr, just as it needs other trace elements against oxidative damage that develops after neurodegenerative trauma. The required amount of Cr is used for the functions of all cells. Nonetheless, the amount of Cr exceeding the required amount triggers the production of ROS, which initiates oxidative damage[19]. The use of trace elements necessary for the body without exceeding the required amount increases their

bioavailability. For example, the amount of Fe required for normal biochemical and physiological functions of the cell can also be used in the treatment of various diseases. However, in a previous study[20], it was reported that an excessive amount of Fe causes oxidative damage to cell building elements, such as lipids, proteins, by triggering the production of highly reactive hydroxyl radicals (Fenton reaction). Like other elements with innate antioxidant and anti-inflammatory abilities (Se, Zn), magnesium has become the center of attention. The Mg mineral, which is a cofactor of many enzymes, contributes to the stability of the cell membrane by reducing the effects of oxidative stres[21].

Current opinions about the mechanism of SCI-secondary damage and the effect of VPA on ion balance and oxidative stress/antioxidant capacity are limited and controversial. This study was designed both to investigate the effects of VPA on SCI and trace elements/antioxidant system and to clarify the current limited and controversial issues.

Materials and Methods

Animal Treatment and Induction of SCI

In total, sixteen male Wistar albino rats (220 to 230 g) were included in this study. The rats were purchased from Veterinary Control Institute Animal Experiments, Elazig, Turkey. To ensure the physiological conditions of the animals, the rats were housed in stainless steel cages under controlled ambient conditions. The humidity was controlled in the range of 40-60% and the temperature was controlled at 18-23°C and maintained at 12:12 h light-dark cycle for 2 days. SCI was created by cross-compression of the aorta for 45 minutes after dissection on the iliac bifurcation in all rats. The rats were randomly divided into two groups of 8 as follows: SCI (control) group [rats were injected with a single dose of normal saline (1.0 ml) intraperitoenally], SCI+VPA group (Depakin® 200 mg/1,000 µL solution, Sanofi Dogu, Istanbul, Turkey, only a single dose of 300 mg/kg of VPA was injected intraperitoneally into rats throughout the experiment).

Anesthesic sedation of all rats before surgery was achieved by intraperitoneally applying a mixture of ketamine hydrochloride (Ketalar, Parke-Davis Eczacıbaşı Eczacıbaşı, Istanbul, Turkey, 50 mg/kg) and xylazine (5 mg/kg, Rompun, Bayer, Istanbul, Turkey). The body temperatures of all rats were measured with a rectal probe inserted into the rectum. Rats received oxygen through a pediatric face mask at a rate of 200 mL per minute during the operation. Following anesthesia sedation, the rats were left in a supine position on special boards for easy surgical intervention, and the operating area was sterilized with 10% povidone-iodine (Betadine, Kensuke, Istanbul, Turkey) solution and closed with a sterile perforated compress to remain open. Then, the intestines revealed by laparotomy incision from the midline of the abdomen were removed from the right side by placing a warm and wet compress. After reaching the retroperitoneal region, the abdominal aorta and the inferior vena cava were isolated after identification and intermittent administration of ketamine hydrochloride was continued to maintain anesthesia. Furtheremore, heparin (Sigma-Aldrich Co., St. Louis, MO, USA, 100 IU/kg) was administered intraperitonally to keep the active coagulation time (ACT) between 200-250 seconds before the abdominal aorta was clamped. To monitor ACT at intervals of 30 minutes peroperatively, Hemochron Jr signature plus device (Keller Medical GmbH, Bad Soden, Germany) was used. Afterwards, an atraumatic microvascular clamp (vascu-statts II, Scanlan Int., St. Paul, MN, USA) was placed along the infrarenal and iliac bifurcation parts of the abdominal aorta for 45 minutes. The operating room temperature was set close to the body temperature value (36.1±0.7°C). During the experiment, aortic systemic blood pressure was maintained at 60-70 mmHg and heart rate at 168.8±22.2 beats/min. When the clamp was removed, the reperfusion was corrected by the reappearance of the lost pulse in the distal aorta. Rats were administered intraperitoneally with protamine sulfate (Sigma Aldrich, St. Louis, MO, USA, 1 mg/kg) to antagonize the effect of heparin and warm sodium lactate solution (Sigma-Aldrich, St. Louis, MO, USA, 10 ml) to control bleeding. In an orderly manner, the abdominal muscles and skin were closed with a 4-0 silk suture. All rats were given cefazolin sodium (Sefazol, Mustafa Nevzat, Istanbul, Turkey) intramuscularly, to prevent possible bacterial infection, and meloxicam subcutaneously (5 mg/kg, Melox Ampoule, Nobel Pharmaceutical Industry, and Trade A.Sh., Istanbul, Turkey). After a 48-hour spinal cord ischemia procedure, all animals were anesthetized (as at the beginning) and sacrificed, and the collected spinal cord samples were washed in ice-cold phosphate-buffered saline, placed in glass bottles, labeled and

frozen in a deep freezer (at -80°C). The frozen sample was weighed and homogenized (Ultra Turrax T25, Janke & Kunkel GmbH & Co., KG, Staufen, Germany) (1:10, w/v) in 100 mmol/litre phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged (5,000 g for 10 min). The supernatant was frozen at -80°C until used for biochemical assays. In this animal experiment study, experimental procedures and all related protocols have been initiated with the prior approval of the Local Ethics Committee for Animal Experiments of the Elazig Veterinary Control Institute (2022/04, EVKEM).

Trace Elements Analysis in the Tissue of Spinal Cord

The element levels of the previously frozen spinal cord samples were first made ready for analysis by the method of burning a pressurized microwave oven (Mars 5, CEM Corporation, ABD, so that the samples become a clear solution without sediment inside), and then determined with the help of an ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry, Vista AX, Varian Inc., Australia) device. Before the measurement, the samples weighed on a delicate scale of about 0.5 g were kept in 10% nitric acid for 24 hours, then washed with distilled water and placed in special propylene tubes of 10 ml and numbered. Then, the combustion process was initiated by adding 10 ml of 65% nitric acid (w/w, Merck, Darmstadt, Germany) to each tube (XP-1500, Tetrafluorometaxyl, CEM Corporation, ABD) and the process was sustained until the desired pressure [150 pounds per square inch (psi)] and temperature (180ºC) were reached. After the combustion process, the cooling process was initiated and the cooled tube samples were transferred to 25 balloon-shaped tubes. The deionized water (resistance: 18 Ω cm-1, P.Nix UP 900, Human Corporation, Seoul, Korea) was added to 25 ml tubes. After that, the tubes were shaken for 10 minutes to homogenize the contents and again transferred to 10 ml propylene tubes and numbered. During the preparation of the spinal cord samples, NIST 1577b (National Institute of Standards & Technology, Gaithersburg, MD, USA) was used as a standard reference material to test the accuracy of the analyses. Before starting the analysis, the ICP-AES device was calibrated with single and multiple element solutions (Merck, Darmstadt, Germany). The Mg, Zn, Se, Cr, Cu and Fe levels of the prepared samples were measured and expressed in µg/g.

Measurement of CAT and GPx Activities and TAS, TOS and OSI Levels in the Spinal Cord Tissue

CAT activity in the spinal cord tissue was performed with the help of Cayma's CAT assay kit (Cayman Chemical Co, Analysis Ann Arbor, MI, USA, Bio-Tek ELx800, Winooski, VT, USA). The experimental procedure is based on the reaction of the enzyme with hydrogen peroxide under methanol catalysis to produce the formaldehyde product. Formaldehyde produced as chromogenic 4-amino-3-hidrazino-5-merkapto-1,2,4-triazol entered and interacted with spectrophotometry (Thermo Scientific Multiskan Spectrum, Ann Arbor, MI, USA) and CAT activity was calculated in terms of U per milligram protein of wet tissue. Cayman's GPx assay kit (Cayman Chemical Co, Ann Arbor, MI, USA) was purchased to detect GPx activity of spinal cord tissue. Briefly, GPx's reduction of hydroperoxides converts reduced glutathione reductase to oxidized glutathione, but the oxidation reaction of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to nicotinamide adenine dinucleotide phosphate ($NADP^+$) causes the oxidized glutathione to convert back to the glutathione reductase form. This condition is observed spectrophotometrically (at 340 nm) as a decrease and it is observed that this decrease is proportional to the GPx activity in the spinal cord. The results were calculated as units per milligram protein (U/mg protein) for spinal cord tissue. Total oxidative status (TOS) and total antioxidant status (TAS) levels were measured on Beckman Coulter AU680 analyzer (Beckman Coulter, Miami, FL, USA) using commercial kits (Rel Assay Diagnostic, Gaziantep, Turkey) based on the method developed by Erel[22]. The experimental procedure of TAS activity in spinal cord tissue is the reduction of the dark blue-green 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ATBS) or ABTS radical to the colorless form of ABTS by antioxidant molecules. As a result, the change in absorption (660 nm) is directly related to the total antioxidant level. Trolox (a water-soluble analogue of vitamin E) was used as a calibrator, and the results obtained were expressed as mmol Trolox Eq/mg protein. The TOS activity method, on the other hand, begins with the oxidants in the sample oxidizing ferrous iono-dianiside complex to ferric ion. The reaction is enhanced by glycerol molecules, which are abundant in the medium. As a result, iron ions form a colored complex in an acidic environment. The color intensity measured spectrophotomet-

rically is related to the total oxidant molecules in the sample. Hydrogen peroxide was used as a calibrator and the results were expressed as μmol H_2O_2 Eq/mg protein. The oxidative stress index (OSI), which is one of the markers of oxidative stress, is found by dividing TOS by TAS. First, the TAS unit, mmol Trolox Eq/mg protein, is converted to the micromol Trolox equivalent/mg protein, and then the OSI is calculated, in short, [OSI = (TOS, μmol H_2O_2 Eq/mg protein)/(TAS, mmol Trolox Eq/mg protein)].

Protein Analysis in the Tissue of Spinal Cord

In this study, a commercial BioRAD DC Protein Assay Kit purchased from San Francisco, CA, USA was used to determine the protein content. Briefly, the purpose of this experiment was to form a blue-colored complex as a result of the reaction of the reagent with the protein. The color intensity of this blue color complex is in parallel with the protein concentration. At the end of the experiment, the protein level was measured by Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, MA, USA) at the absorption wavelength (595 nm).

Behaviors Evaluation

Motor function recovery of all rats was estimated using the Basso, Beattie and Bresnahan (BBB) scoring method[23] with a 21-point open-space locomotor scale from 0 (indicating a state of complete paralysis) to 21 (indicating normal motor function). After SCI, at the 1st, 6th, 12th, 24th and 48th hours, the rats' movements were recorded by two blind observers in groups. The movements of both hind legs, walking skills, stability, trunk, tail and paw position, body coordination and finger span of the rats were observed and recorded. Thus, the desired BBB score was obtained by taking the average of the recorded scores. For the inclined plane test[24], the rats were placed on a tester with a five-stage tilt (a rubber mat with horizontal ridges 3 mm apart, fixed to a flat board). Initially horizontally (0°), and after each procedure, the upward angle was increased by 5-10°, and thus the maximum angle that a rat could maintain for 5 seconds without falling until it lost its grip was recorded, and the average score of each rat was obtained.

Statistical Analysis

In this study, SPSS v. 21.0 (Statistical Package for the Social Sciences; IBM Corp., Armonk, NY, USA) statistical analysis program was used. The experimental data obtained are indicated as the mean±standard error of the mean for 8 rats in each group. One-way analysis of variance (ANOVA) was used for biochemical data, and the Tukey post-hoc option was used to assess the changes between the groups. *p*-value below 0.05 was considered statistically significant.

Results

Effect of VPA on TOS and OSI Levels in the Spinal Cord Tissue

The study shows the findings of the relationship between the level of oxidative stress (as TOS and OSI) in the spinal cord tissue and the secondary damage induced by SCI. In addition, the application of VPA after experimental SCI revealed that a decrease in the levels of TOS and OSI compared to the SCI (control) group ($p<0.05$) may be a consequence of the neuroprotective property of VPA (Figures 1 and 2).

Effect of VPA on TAS Level and CAT and GPx Activities in the Spinal Cord Tissue

Compared to the SCI (control) group, TAS level, CAT and GPx activities increased significantly in the SCI+VPA group ($p<0.05$, Figures 3-5). In SCI rats, the decrease of TAS level and CAT and GPx activities due to damage was prevented with the administration of VPA. This situation has shown that it is a result of the effect of VPA on increasing antioxidant activity.

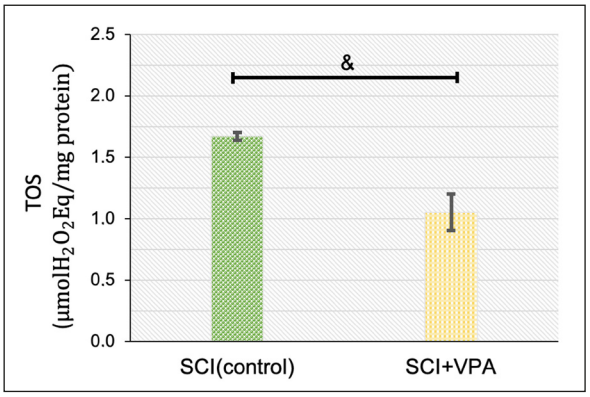

Figure 1. The level of total oxidative status (TOS) in the spinal cord tissue (μmol H_2O_2 Eq/mg protein). The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. $^{\&}p<0.05$ against the SCI (control) group.

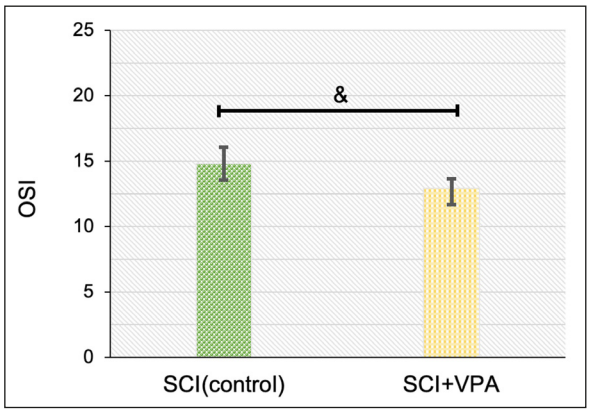

Figure 2. The level of oxidative stress indices (OSI) in the spinal cord tissue. The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. $^{\&}p < 0.05$ against the SCI (control) group.

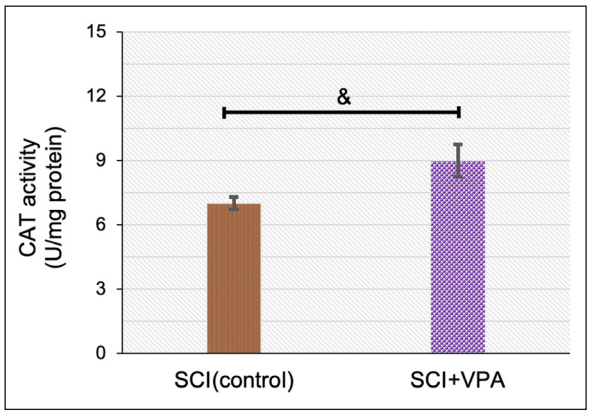

Figure 4. The activity of catalase (CAT) in the spinal cord tissue (U/mg protein). The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. $^{\&}p < 0.05$ against the SCI (control) group.

Effect of VPA on Trace Element Levels in the Spinal Cord Tissue

In the SCI+VPA group levels compared to the SCI (control) group, a significant increase in spinal cord tissue Mg, Zn and Se were observed (Figure 6). Administration of VPA to rats with SCI prior to SCI-induced secondary injury significantly improved Mg, Zn and Se values by reducing secondary injury. Figure 7 shows the findings obtained regarding the Cr, Cu and Fe levels in spinal cord tissues. Cr, Cu and Fe levels in the SCI (control) group were statistically higher than those in the treatment group. However, VPA treatment of SCI rats before SCI-induced secondary damage reduced the effect of secondary damage and prevented the excessive increase in Cr, Cu and Fe levels.

Neurological Function Assessment

As shown in Figure 8A, compared with the rats in the SCI (control) group, the rats with

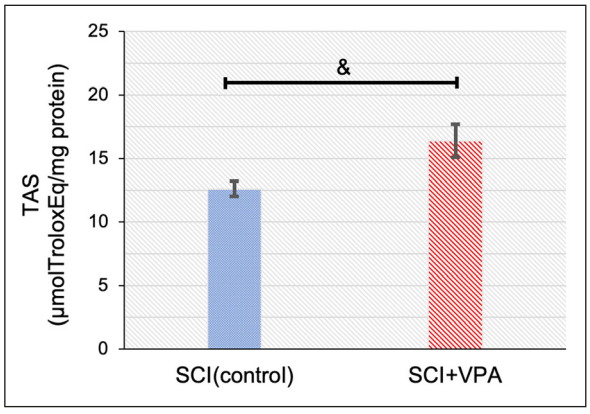

Figure 3. The level of total antioxidant status (TAS) in the spinal cord tissue (µmolTtroloxEq/mg protein). The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. $^{\&}p < 0.05$ against the SCI (control) group.

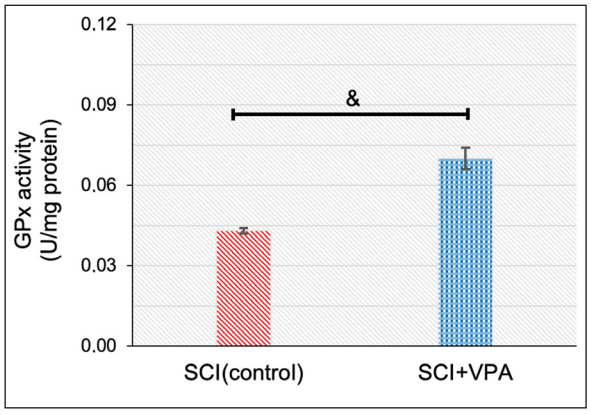

Figure 5. The activity of glutathione peroxidase (GPx) in the spinal cord tissue (U/mg protein). The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. $^{\&}p < 0.05$ against the SCI (control) group.

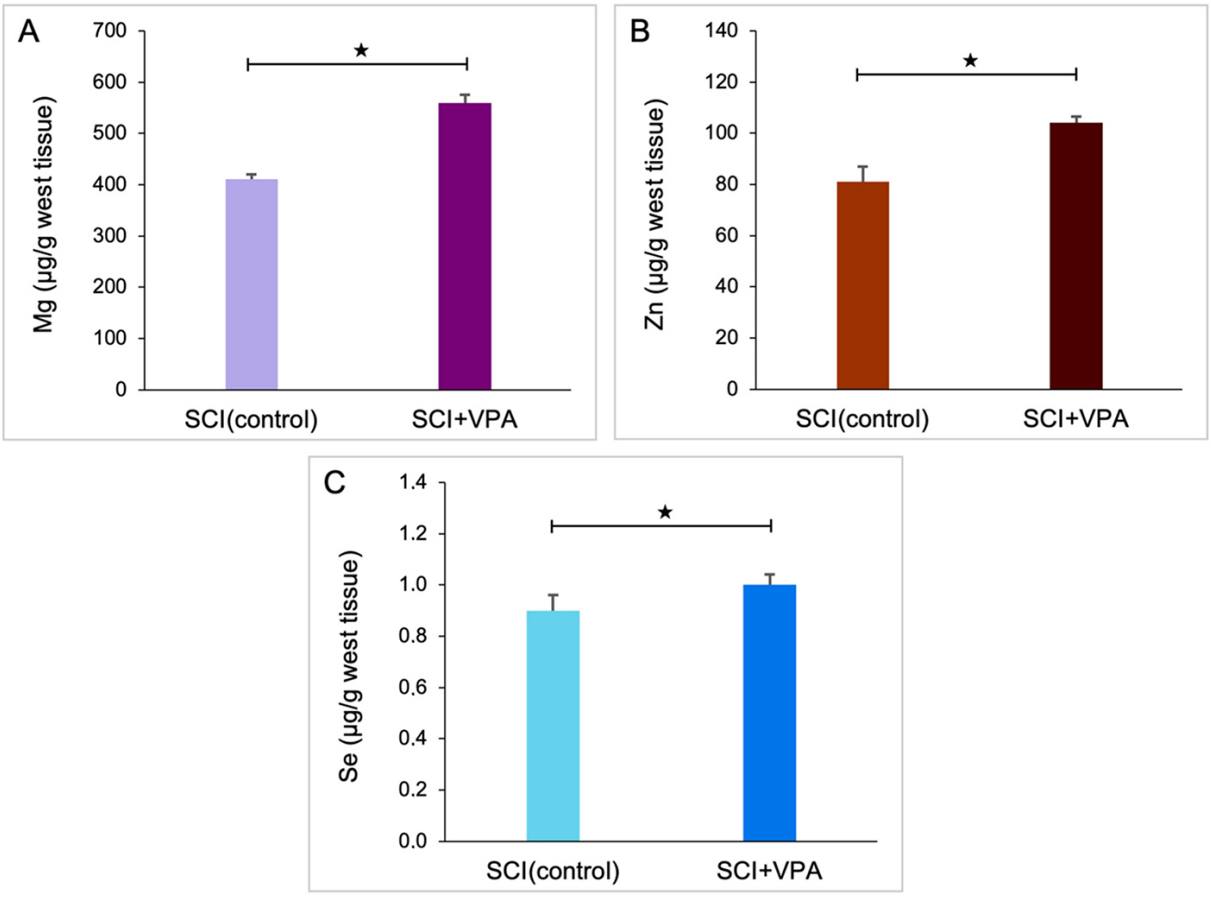

Figure 6. **A**, Magnesium (Mg), (**B**), zinc (Zn), (**C**), selenium (Se) levels spinal cord damage and the levels of essential elements in the spinal cord tissue after the administered VPA. The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. ★$p < 0.05$ against the SCI (control) group.

SCI+VPA showed higher BBB scores at each time point, and the differences were statistically significant ($p<0.05$). It was shown that there was a significant improvement in neurology function. The BBB scores of the SCI rats at 48 hours remained <10 points, whereas the rats treated with VPA had scores above 10 points, and the differences were statistically significant ($p<0.05$). As shown in Figure 8B, giving VPA following SCI, the angle of incline of the rats was increased, compared with those in the SCI (control) group at each time point, and the differences were statistically significant ($p<0.05$). These results revealed that only SCI weakened the neurological function of rats, and the administration of VPA following SCI accelerated the recovery of neurological function.

Discussion

Our experimental results have shown that oxidative stress due to SCI-secondary damage causes a decrease in both elemental levels (Mg, Zn and Se) and antioxidant enzyme activity (TAS, CAT and GPx). In addition, observation of increase in the levels of TOS, OSI, Cr, Cu and Fe revealed that the severity of the damage is multifaceted. In particular, the administration of VPA to rats with SCI before the onset of the actual effect of SCI-induced oxidative stress is important in terms of minimizing this effect. Despite limited in number, the recent researches[25] on the neuroprotective effect of VPA due to its anti-oxidation and anti-inflammatory properties have opened a new path in the treatment of neurological diseases.

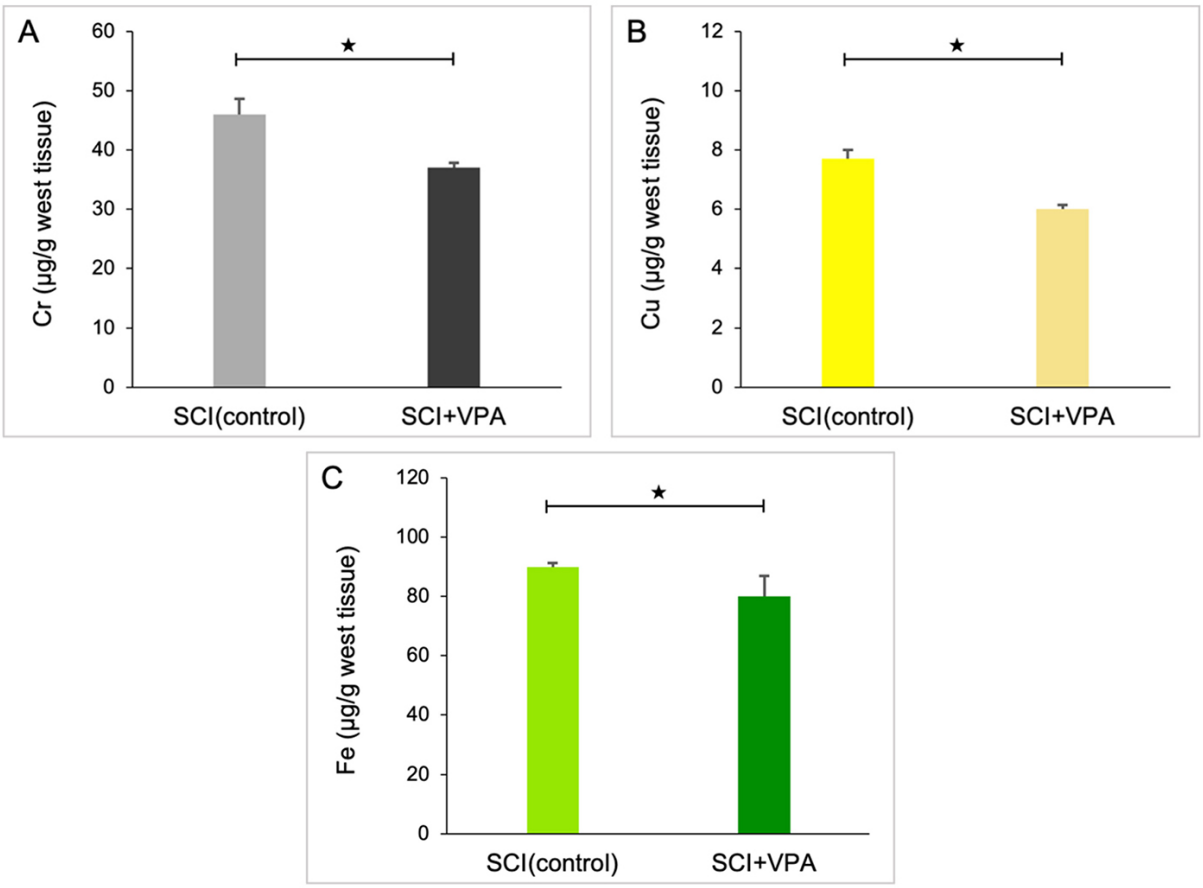

Figure 7. **A**, Chromium (Cr), (**B**), copper (Cu), (**C**), iron (Fe) levels spinal cord damage and the levels of essential elements in the spinal cord tissue after the administered VPA. The experimental data obtained are indicated as the mean ± standard error of the mean for 8 rats in each group. For biochemical data, one-way analysis of variance (ANOVA) was used; differences between the groups were measured using the Tukey post-hoc option. *p*-value below 0.05 was considered statistically significant. ★$p<0.05$ against the SCI (control) group.

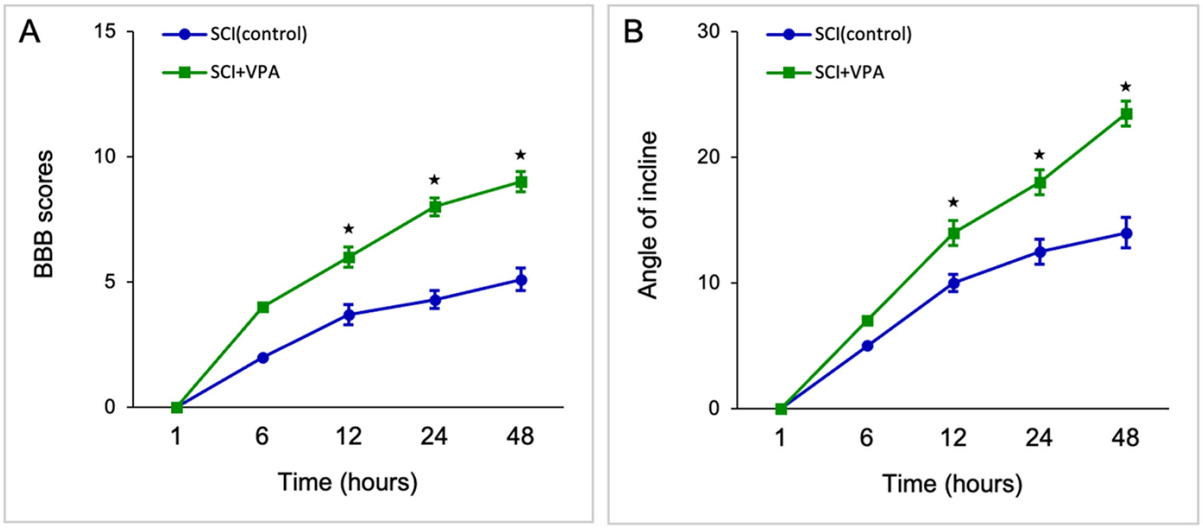

Figure 8. Results of the motor function assessment of the rats. **A**, BBB scores. **B**, Angle of incline. ★$p<0.05$ *vs.* SCI+VPA group. SCI, spinal cord injury; VPA, valproic acid; BBB, Basso, Beattie, Bresnahan.

Accordingly, many opinions have been put forward about the mechanisms underlying the antioxidant property of VPA. For example, one of these views[5] is that VPA acts as an epigenetic regulator by inhibiting histone deacetylases, increasing histone acetylation, inducing DNA and histone methylation association and gene expression, and influencing chromatin formation. Since extreme ROS production damages many cellular components, including DNA, this mechanism is very important in terms of both chromatin structure and maintenance of gene regulation. In addition, one of the important benefits of VPA is that it has an antioxidant effect against glutamate-induced excitotoxicity, which plays a role in the pathophysiology of some neurological diseases, including mitochondrial cellular damage[26]. A recent animal experiment suggests that VPA significantly promotes neurogenesis after traumatic brain injury by increasing BDNF (as a critical mediator of neuronal plasticity), thereby significantly reducing brain injury status[27]. On the other hand, clinically, it was reported[28] that the serum total antioxidant capacity of epileptic children receiving VPA monotherapy was much better than the children receiving monotherapy with some different epileptic agents. Moreover, *in vivo* studies[29] show that administration of VPA to alloxane-induced diabetic rats with hepatopathy not only significantly reduced ROS-mediated lipid peroxidation, blood lipids, and liver parameters, but also significantly increases the antioxidant activity of SOD and GPx. Based on all this information given, it is noteworthy that the neuroprotection of VPA is associated with its anti-inflammation, anti-oxidation properties and antioxidant potentiating effects. The results of this study reveal that the final treatment of rats with VPA after experimental SCI significantly reduced secondary damage-mediated oxidative stress in the treatment group, and the result of this study is consistent with the above-mentioned research. Traumatic-SCI (applications such as temporary vascular clamping) is an important phenomenon as it causes ischemia due to impaired blood flow and low anaerobic metabolic capacity. The ROS produced due to blood supply to the tissue (reperfusion) following the ischemia may cause triggering of oxidative stress, multiple oxidant mechanisms, inflammation and increase in apoptotic mechanism, as well as tissue damage[30]. In a previous study[31], the decrease in antioxidant capacity of the spinal cord, which is affected by ischemia-induced ROS-related events, was thought to be related to the repair of the damage. As a matter of fact, polyunsaturated fatty acid (PUFA) is the main component of the cell membrane, and the presence of bis-allylic methylene groups in its structure has made it very sensitive to ROS and nitrogen, making it the first target of attack. As a result, events such as ion imbalance triggered by the disruption of ion transport after membrane permeability are very important, as they lead to cell death. It has been reported[32] that the spinal cord is particularly prone to ROS attack due to excessive PUFA content, high oxygen consumption, high Fe content and poor antioxidant defense properties. In another study[33], they reported that differentially expressed genes (DEGs) can play important roles in the development of SCI according to functional enrichment analysis. Our current data reveal that treating rats with VPA after SCI before the secondary damage had spread significantly increased the antioxidant activity of TAS, CAT and GPx. We have seen in our study findings that one of the positive effects of VPA on spinal cord injury during SCI-secondary injury is responsible for the increase in antioxidant enzyme activity. Because of the highly active neurochemical events, the need for macro-micro nutrients of neural organs such as the spinal cord is very crucial. Similarly, understanding the trace element needs and elemental balance of SCI patients is important in terms of the etiology of the disease[34]. At the same time, current experimental and clinical research is not enough to reveal the imbalance of trace elements in organ tissues, for reasons such as SCI[35]. A recent study[36] showed that ion imbalance is the most important factor responsible for ischemia-induced brain death compared to oxidative stress and inflammation. Basically, ion imbalance is known to occur a short time after the ischemic event. This means that ionic exchange is the most important factor responsible for neuron death in the ischemia process. Thus, understanding the underlying events of trace element imbalance during the ischemic event induced by SCI-secondary damage, providing clarification of the relationship of changes in these elements with SCI will contribute to the understanding of the pathophysiology of neurodegenerative diseases.

The trace element Zn is an essential nutrient for the body, as it is used in various physiological and biochemical functions, and has a role in many processes, such as oxidative stress, inflammation, wound healing, and DNA damage repair. In addition, it is an important phenomenon that Zn ion is located in many tissues, especially in the spinal

cord, brain, muscle, liver and bones, and connects to more than 300 enzymes and more than 2,000 transcription factors for various biochemical pathways and cellular functions[37]. Some evidence has shown that an increase in the level of Zn in the ischemic cerebral cortex leads to an increase in SOD and CAT activities, and there is a positive relationship between the level of Zn and the activity of antioxidant enzymes[38]. In other studies[39], they have reported that Zn supplementation in ischemic/reperfusion models of various organs increases the levels of SOD, CAT, GPx, and nuclear factor erythroid 2 (Nrf2) but decreases the level of MDA, so they have also reported that Zn supplementation to prevent ischemia/reperfusion damage during surgery or trauma will contribute to recovery. According to some experimental studies[40], Zn supplementation to rats contributes to the healing of injured spinal cord tissue, as it causes regulation of BDNF expression and inhibition of HDACs. Also, a beneficial property of Zn ion is to prevent glutamate neurotoxicity by modulating glutamate signaling. As a consequence of this, it is very important for neuron health in that it eliminates the cause of many neurological diseases. In this study, SCI-secondary damage causes a significant decrease in the level of Zn and increased oxidative stress. However, the administration of VPA to rats after SCI significantly reversed these negative findings. Our findings revealed that the positive effect of VPA on the level of Zn is a consequence of its neuroprotective property.

Mg, one of the intracellular basic cations of a living organism, is involved in a large number of important stages of cellular and biochemical events. For example, it includes various functions such as acting as a cofactor in many enzymatic reactions, synthesizing DNA and proteins, inhibiting oxidative stress, maintaining neuronal functions. One of the neurological functions of Mg is to prevent the formation of glutaminergic signals that lead to neurotoxicity due to the blocking of the calcium channel at the n-methyl-D-aspartate (NMDA) receptor. In addition, a study finding[41] indicated that Mg plays an important role in neurological recovery during cerebral ischemia by preventing ROS-induced lipid peroxidation. Therefore, Mg deficiency can lead to both excessive NMDAR activation and lipid peroxidation, slowing neuronal healing. In this study, SCI caused a decrease in the level of Mg and the spread of oxidative stress in the affected spinal cord. Remarkably, it was observed that Mg levels increased with VPA administration to rats in the SCI group before the secondary damage spread. In an *in vivo* study[42], it is revealed that there is an inversely proportional relationship between the Mg ion level and ROS-induced lipid peroxidation during the period of secondary damage induced by SCI. Our findings, which are parallel to the information given above, show that the neuroprotective mechanism of VPA during SCI is to provide an increase in the Mg level to suppress ROS-mediated oxidative stress, which is the greatest indicator of secondary damage to the damaged spinal cord.

Selenium ion has important effects that are open to many innovations, including regulation of the oxidant/antioxidant system, anti-oxidation, anti-inflammation, anti-carcinogenic effects. Conversely, decreased Se levels disrupt the oxidant/antioxidant balance, making antioxidant defense powerless, which leads to the spread of oxidative damage and further cellular death[43]. Similarly, in our study, low Se level caused by SCI triggered oxidative damage to the affected spinal cord, which caused the damage to spread further; accordingly, it can be concluded that this condition is related to a decrease in its antioxidant power in proportion to a decrease in the Se level.

The best known antioxidant activity of the Se ion is that it inactivates lipid peroxides as part of the structure of different types of GPx. In one study[44], it was reported that Se may have protective effects against kidney damage caused by ischemia/reperfusion. In another study[45], they reported that Se supplementation increased SOD, CAT, and GPx activities as well as reduced MDA levels in rat models with ovarian ischemia/reperfusion injury. Therefore, Se supplementation can contribute to recovery by preventing possible ischemia/reperfusion damage during surgery or trauma. These supplements are quite important, as they benefit the fulfillment of radical scavenging functions as part of the antioxidant system. In a clinical study[46], it was found that any increase in neurotoxically oriented neuron degenerations is prevented by administering Se to epileptic patients, thereby causing a decrease in epileptic seizures and subsequent improvement in neuronal damage. In this study, we observed that VPA, which was given as a protector of neuronal damage, significantly increased the level of Se in the spinal cord after SCI. These observations suggest that VPA can prevent SCI-induced oxidative stress of spinal cord cells by increasing the Se level.

The amount of Cr at a normal level is sufficient for cellular and biochemical functions in the body. However, it is important in terms of ion toxicity to reveal that the amount of Cr exceeding the normal level increases the production of ROS, which causes the formation of oxidative damage. Briefly, it is obvious that the adjustment of the elemental balance or the required Cr level is an event that plays an important role not only in maintaining cellular and biochemical functions, but also in preventing ROS-induced oxidative damage that damages cells. Meanwhile, the role of oxidative stress in the change of both Cr level and Cr metabolism is of great importance in terms of the pathophysiology of events such as ischemia. In addition, due to the reduction of Cr (III) to Cr (II), an excessive amount of Cr (II) can spontaneously participate in the reaction with hydrogen peroxide and produce highly reactive hydroxyl radicals. The increase in lipid peroxidation caused by these reactions has been confirmed by research[47]. On the other hand, it was first shown in a study[48] that 100% increased Cr level in the study of cerebral ischemia-reperfusion in gerbil can increase the level of MDA, which is an indicator of oxidative damage. Similarly, our current finding shows that SCI-secondary damage leads to an excessive increase in the amount of Cr in the spinal cord. However, we have determined that in rats with SCI, VPA administration significantly reduces the amount of excessive Cr before the secondary damage has fully spread. We believe that this beneficial effect of VPA is probably due to both its chelating and neuroprotective properties, namely that it reduces oxidative stress caused by excessive Cr levels during SCI, ensuring that appropriate and necessary Cr levels are maintained.

The element Fe, which is required 3-5 G for an adult individual, is very important for the cellular and biochemical functions of the body. In one study[49], it was shown that excessive Fe levels trigger oxidative stress after disturbing the ROS balance, causing various adverse events such as neurodegeneration, DNA-induced mutations, and cytotoxic reactions. In addition, ischemia reperfusion-induced ROS can spontaneously react with Fe to produce highly reactive hydroxyl radicals with its participation in the Fenton reaction, causing more oxidative organ damage and even Fe-induced cell death (ferroptosis). Recent studies[34] have reported that some epileptic drugs will have effect of preventing ion imbalance or excessive accumulation of ions by restoring the imbalance of antioxidant redox systems in the nervous organs. In our study, it was shown that the administration of VPA to rats with SCI before SCI-secondary damage significantly balanced the excessive amount of Fe in the damaged spinal cord. Our findings are consistent with the research findings given above. In summary, the fact that VPA eliminates radicals participating in the fenton reaction with its neuroprotective effect and prevents excess Fe amount with its chelation ability confirms our research purpose.

Appropriate levels of Cu are involved in a variety of cellular functions, including the synthesis of many neural structures. Various pathological conditions or other causes cause a significant increase in Cu level, similar to Fe. The increased level of Cu is quite harmful for cells, because it participates in the Fenton reaction for the production of highly reactive hydroxyl radicals, and thus, causes further oxidative stress, and subsequent cell death. In different experimental studies[50], it has been reported that excessive Cu accumulation in the affected brain tissue after cerebral ischemic events occurs also in different tissues. In a clinical study[51] of epileptic children, increase in the plasma Cu level and decrease in the Zn level reveal the existence of a relationship between the etiology of epilepsy and the level of trace elements. Recently, the discovery that antiepileptic drugs have many beneficial properties, except for the treatment of epilepsy, has attracted the attention of many researchers[35]. In our study, we showed that the administration of VPA to rats with SCI before SCI-secondary damage significantly balanced the excessive level of Cu in the damaged spinal cord. Our findings are consistent with the research findings mentioned above. In short, the fact that VPA destroys the radicals participating in the fenton reaction with its neuroprotective effect and prevents the increase of excess Cu level with its chelation ability confirms our research purpose.

According to many research results[52], among the most clinically important indicators of SCI are motor and sensory complications, which lead to many negative effects. The BBB locomotor rating scale and Rivlin's inclined plane test are the most important methods used to assess the movement recovery of experimental animals after SCI[53]. The results of this study showed that BBB scores and oblique plate angles decreased in the SCI (control) group. In addition, the administration of VPA to SCI rats reversed these negative effects. These results showed that the experimental SCI model was properly constructed and that VPA contributes to the improvement of neurological function by preventing SCI-induced secondary damage.

Despite many negative opinions about the neuroprotective properties of VPA, the fact that it has beneficial properties in recent times has been supported by this study. VPA is important in terms of its neuroprotective property to protect damaged spinal cord tissue by activating various positive mechanisms. This study is the first to confirm that VPA regulates the levels of essential elements in the spinal cord during SCI. In conclusion, our findings confirmed that VPA is a promising agent with neuroprotective and other beneficial properties, balancing the excessive accumulation of elements associated with oxidative stress and Fenton reaction, increasing antioxidant enzyme activity and antioxidant element levels at the appropriate level. In summary, after events such as SCI, we think that further research is needed to clarify the beneficial roles of VPA on both trace element level and antioxidant enzyme activity.

Conclusions

Our results show that the application of VPA has positive effects on oxidative stress, antioxidant and trace element level after secondary damage caused by SCI. The application of VPA to prevent SCI damage can be used as an adjunctive treatment option as well as the existing clinical treatment, and however, more research is needed in terms of the use of different combinations of VPA-like agents in the clinical treatment of SCI.

Authors' Contributions

Conceptualization, M.U.; methodology, M.U.; software, M.U.; validation, M.U., formal analysis, M.U. and O.G.A.; investigation, M.U.; resources, M.U. and O.G. A.; data curation, M.U.; writing-original draft preparation, M.U.; writing-review and editing, M.U.; visualization, M.U. and O.G.A.; supervision, M.U.; project administration, M.U.; funding acquisition, O.G.A.

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Ethics Approval

The Animal Experimentation Ethics Committee accepted the research at the Veterinary Control Institute Animal Experiments, Elazig, Turkey (No. EVKEM 2022/04).

Data Availability

All data are included within the article.

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Conflicts of Interest

The Authors declare that there are no conflicts of interest.

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References

1) Scheijen EEM, Hendrix S, Wilson DM III, Oxidative DNA Damage in the Pathophysiology of Spinal Cord Injury: Seems Obvious, but Where Is the Evidence? Antioxidants 2022; 11: 1728.
2) Anjum A, Yazid MD, Fauzi Daud M, Idris J, Ng AMH, Selvi Naicker A, Ismail OHR, Athi Kumar RK, Lokanathan Y. Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. Int J Mol Sci 2020; 21: 7533.
3) Kim K, Li Y, Jin G, Chong W, Liu B, Lu J, Lee K, Demoya M, Velmahos GC, Alam HB. Effect of valproic acid on acute lung injury in a rodent model of intestinal ischemia reperfusion. Resuscitation 2012; 83: 243-248.
4) Sher Y, Cramer A, Ament A, Lolak S, Maldonado JR. Valproic Acid for Treatment of Hyperactive or Mixed Delirium: Rationale and Literature Review. Psychosomatics 2015; 56: 615-625.
5) Rouaux C, Panteleeva I, René F, Gonzalez de Aguilar JL, Echaniz-Laguna A, Dupuis L, Menger Y, Boutillier AL, Loeffler JP. Sodium valproate exerts neuroprotective effects in vivo through CREB-binding protein-dependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. J Neurosci 2007; 27: 5535-5545.
6) Fuentealba CR, Fiedler JL, Peralta FA, Avalos AM, Aguayo FI, Morgado-Gallardo KP, Aliaga EE. Region-Specific Reduction of BDNF Protein and Transcripts in the Hippocampus of Juvenile Rats Prenatally Treated With Sodium Valproate. Front Mol Neurosci 2019; 12: 261.
7) Havelikova K, Smejkalova B, Jendelova P. Neurogenesis as a Tool for Spinal Cord Injury. Int J Mol Sci 2022; 23: 3728.
8) Naseh M, Bayat M, Akbari S, Vatanparast J, Shabani M, Haghighi AB, Haghani M. Neuroprotective effects of sodium valproate on hippocampal cell and volume, and cognitive function in a rat model of focal cerebral ischemia. Physiol Behav 2022; 251: 113806.
9) Ding LZ, Xu J, Yuan C, Teng X, Wu QM. MiR-7a ameliorates spinal cord injury by inhibiting neuronal apoptosis and oxidative stress. Eur Rev Med Pharmacol Sci 2020; 24: 11-17.

10) Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. Neuropharmacology 2008; 55: 310-318.
11) Shultz RB, Zhong Y. Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. Neural Regen Res 2017; 12: 702-713.
12) Jia Z, Zhu H, Li J, Wang X, Misra H, Li Y. Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord 2012; 50: 264-274.
13) Cavus G, Altas M, Aras M, Ozgür T, Serarslan Y, Yilmaz N, Sefil F, Ulutas KT. Effects of montelukast and methylprednisolone on experimental spinal cord injury in rats. Eur Rev Med Pharmacol Sci. 2014;18: 1770-1777.
14) Ulas, M, Cay, M. Effects of 17β-Estradiol and Vitamin E Treatments on Blood Trace Element and Antioxidant Enzyme Levels in Ovariectomized Rats. Biol Trace Elem Res 2011; 139: 347-355.
15) Woźniak B, Woźniak A, Mila-Kierzenkowska C, Kasprzak HA. Correlation of oxidative and Antioxidative processes in the blood of patients with cervical spinal cord injury. Oxidative Med Cell Longev 2016; 2016: 6094631.
16) Hesamian MS, Eskandari N. Potential role of trace elements (Al, Cu, Zn, and Se) in multiple sclerosis physiopathology. NeuroImmunoModulation 2020; 27: 163-177.
17) Galli F, Battistoni A, Gambari R, Pompella A, Bragonzi A, Pilolli F, Iuliano L, Piroddi M, Dechecchi MC, Cabrini G. Working Group on Inflammation in Cystic Fibrosis. Oxidative stress and antioxidant therapy in cystic fibrosis. Biochim Biophys Acta 2012; 1822: 690-713.
18) Palmieri B, Sblendorio V. Oxidative stress tests: overview on reliability and use. Part I. Eur Rev Med Pharmacol Sci 2007; 11: 309-342.
19) Singh V, Singh N, Verma M, Kamal R, Tiwari R, Sanjay Chivate M, Rai S.N, Kumar A, Singh A, Singh MP, Vamanu E, Mishra V. Hexavalent-Chromium-Induced Oxidative Stress and the Protective Role of Antioxidants against Cellular Toxicity. Antioxidants 2022; 11: 2375.
20) Lee JC, Son YO, Pratheeshkumar P, Shi X. Oxidative stress and metal carcinogenesis. Free Radic Biol Med 2012; 53: 742-757.
21) Fiorentini D, Cappadone C, Farruggia G, Prata C. Magnesium: biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. Nutrients 2021; 13: 1136.
22) Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37: 277-285.
23) Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995; 12: 1-21.
24) Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg 1977; 47: 577-581.
25) Yu SH, Cho DC, Kim KT, Nam KH, Cho HJ, Sung JK. The neuroprotective effect of treatment of valproic Acid in acute spinal cord injury. J Korean Neurosurg Soc 2012; 51: 191-198.
26) Zaky A, Mahmoud M, Awad D, El Sabaa BM, Kandeel KM, Bassiouny AR. Valproic acid potentiates curcumin-mediated neuroprotection in lipopolysaccharide induced rats. Front Cell Neurosci 2014; 21: 337.
27) Dedoni S, Marras L, Olianas MC, Ingianni A, Onali P. Downregulation of TrkB expression and signaling by valproic acid and other histone deacetylase inhibitors. J Pharmacol Exp Ther 2019; 370: 490-503.
28) Beltrán-Sarmiento E, Arregoitia-Sarabia CK, Floriano-Sánchez E, Sandoval-Pacheco R, Galván-Hernández DE, Coballase-Urrutia E, Carmona-Aparicio L, Ramos-Reyna E, Rodríguez-Silverio J, Cárdenas-Rodríguez N. Effects of Valproate Monotherapy on the Oxidant-Antioxidant Status in Mexican Epileptic Children: A Longitudinal Study. Oxid Med Cell Longev 2018; 4: 10-13.
29) Akindele AJ, Otuguor E, Singh D, Ota D, Benebo AS. Hypoglycemic, antilipidemic and antioxidant effects of valproic acid in alloxan-induced diabetic rats. Eur J Pharmacol 2015; 762: 174-183.
30) Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012; 298: 229-317.
31) Zhang RF, Zeng M, Lv N, Wang LM, Yang QY, Gan JL, Li HH, Yu B, Jiang XJ, Yang L. Ferroptosis in neurodegenerative diseases: inhibitors as promising candidate mitigators. Eur Rev Med Pharmacol Sci 2023; 27: 46-65.
32) Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 2012; 1822: 675-684.
33) Chen XJ, Mou XQ, Zou YG, Peng ZY, Yang JX. Screening of key genes associated with contused rat spinal cord with DNA microarray. Eur Rev Med Pharmacol Sci 2013; 17: 2949-2955.
34) Wang HD, Wei ZJ, Li JJ, Feng SQ. Application value of biofluid-based biomarkers for the diagnosis and treatment of spinal cord injury. Neural Regen Res 2022; 17: 963-971.
35) Nazıroğlu M, Yürekli VA. Effects of antiepileptic drugs on antioxidant and oxidant molecular pathways: Focus on trace elements. Cell Mol Neurobiol 2013; 33: 589-599.
36) Babayan LA, Hayrapetyan HA, Gulyan AK, Danoyan HE, Vardanyan HA, Gasparyan NA, Sarafyan PK, Grigoryan SG, Harutyumyan TG. Influence of hydrometeorological indices on electrolytes and trace elements homeostasis in patients with ischemic heart disease. Int J Biometeorol 2020; 64: 2171-2176.
37) Marchan R, Cadenas C, Bolt HM. Zinc: as a multipurpose trace element, Arch Toxicol 2006; 80: 1-9.
38) Lin MC, Liu CC, Lin YC, Hsu CW. Epigallocatechin Gallate Modulates Essential Elements, Zn/Cu Ratio, Hazardous Metal, Lipid Peroxidation, and Antioxidant Activity in the Brain Cortex during Cerebral Ischemia. Antioxidants 2022; 11: 396.
39) Akbari G. Role of zinc supplementation on ischemia/reperfusion injury in various organs. Biol Trace Elem Res 2020; 196: 1-9.
40) Baltan S, Morrison RS, Murphy SP. Novel protective effects of histone deacetylase inhibition on stroke and white matter ischemic injury. Neurotherapeutics 2013; 10: 798-807.

41) Kirkland AE, Sarlo GL, Holton KF. The role of magnesium in neurological disorders. Nutrients 2018; 10: 730.
42) Sperl A, Heller RA, Biglari B, Haubruck P, Seelig J, Schomburg L, Bock T, Moghaddam A. The Role of Magnesium in the Secondary Phase After Traumatic Spinal Cord Injury. A Prospective Clinical Observer Study. Antioxidants 2019; 8: 509.
43) Kuršvietienė L, Mongirdienė A, Bernatonienė J, Šulinskienė J, Stanevičienė I. Selenium Anticancer Properties and Impact on Cellular Redox Status. Antioxidants 2020; 9: 80.
44) Wang S, Chen Y, Han S, Liu Y, Gao J, Huang Y, Sun W, Wang J, Wang C, Zhao J. Selenium nanoparticles alleviate ischemia reperfusion injury-induced acute kidney injury by modulating GPx-1/NLRP3/Caspase-1 pathway. Theranostics 2022; 12: 3882-3895.
45) Liu L, Liu C, Hou L, Lv J, Wu F, Yang X, Ren S, Ji W, Wang M, Chen L. Protection against ischemia/reperfusion induced renal injury by cotreatment with erythropoietin and sodium selenite. Mol Med Rep 2015; 12: 7933-7940.
46) Wang M, Zhang X, Jia W, Zhang C, Boczek T, Harding M, Liu Y, Li M, Zhang S, Lei S, Zhang D, Guo F. Circulating glutathione peroxidase and superoxide dismutase levels in patients with epilepsy: A meta-analysis. Seizure 2021; 91: 278-286.
47) Casalegno C, Schifanella O, Zennaro E, Marroncell S, Briant R. Collate literature data on toxicity of Chromium (Cr) and Nickel (Ni) in experimental animals and humans. Supporting Publications 2015; 12: 287.
48) Fang KM, Cheng FC, Huang YL, Chung SY, Jian ZY, Lin MC. Trace element, antioxidant activity, and lipid peroxidation levels in brain cortex of gerbils after cerebral ischemic injury. Biol Trace Elem Res 2013; 152: 66 74.
49) Galaris D, Barbouti A, Pantopoulos K. Iron homeostasis and oxidative stress: An intimate relationship, Biochim. Biophys. Acta Mol Cell Res 2019; 1866: 118535.
50) Gaetke LM, Chow-Johnson HS, Chow CK. Copper: toxicological relevance and mechanisms. Arch Toxicol 2014; 88: 1929-1938.
51) Talat MA, Ahmed A, Mohammed L. Serum levels of zinc and copper in epileptic children during long-term therapy with anticonvulsants. Neurosciences 2015; 20: 50336.
52) Hagen EM. Acute complications of spinal cord injuries. World J Orthop 2015; 6: 17-23.
53) Schumacher PA, Siman RG, Fehlings MG. Pretreatment with calpain inhibitor CEP-4143 inhibits calpain I activation and cytoskeletal degradation, improves neurological function, and enhances axonal survival after traumatic spinal cord injury. J Neurochem 2007; 4: 1646-1655.