



Valproic Acid Attenuates Oxidative Damage in Rat Spleen Tissue Induced By Spinal Cord Damage[#]

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ABSTRACT

Events such as oxidative stress caused by spinal cord injury (SCI) are a serious condition because they negatively affect many organs. Alternative treatment options for this type of injury are quite limited. In this study, we planned to investigate the effect of oxidative damage on the spleen tissue of rats with spinal cord damage and the protective role of valproic acid (VPA) in this damage. Sixteen Wistar albino rats were divided into two equal groups. No treatment was administered to the rats in Group 1 (SCI-(Control)), but a single dose of 300 mg/kg intraperitoneally VPA was administered to the rats in Group 2 (SCI-VPA). Superoxide dismutase (SOD) activities, total antioxidant status (TAS) and total oxidant status (TOS) levels were examined as markers of oxidative stress in spleen tissues taken after decapitation of rats. VPA treatment increased the SOD and TAS level but decreased the TOS level, indicating improved oxidative damage and impaired enzymatic antioxidant levels in spleen tissue homogenate damaged by SCI. We have observed that VPA, which has many beneficial properties, has a significant healing effect on spleen tissue affected by SCI-induced oxidative stress.

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Introduction

Spinal cord injury (SCI) causes dysfunction and damage to many organs (such as the spleen), especially due to damage to the autonomic system and restriction of blood flow. The appearance of primary damage after the formation of SCI does not spread the disease, except for local symptoms. However, secondary damage following primary damage is important from the point of view of SCI epidemiology, since its effect is observed in almost all organs, spreading the disease further (Sun et al., 2016). Secondary damage involves many different physiopathological mechanisms. For example, one of these mechanisms is hypoxia, which is caused by compression of the vessel, followed by the appearance of ischemia. The damage caused by ROS to many structures in the cells, which occurs after the blood flow to the tissues is interrupted by ischemia, increases the extent of the disease (Kalogeris et al., 2012). In addition, SOD, which forms the first enzymatic line of defense in the body, converts both ROS and superoxide radicals into less reactive H₂O₂ and O₂, which can reduce the secondary damage that will

develop after SCI (Villa et al., 2019). After SCI, an increase in TOS levels, which are important markers of oxidative stress due to ROS, and a decrease in TAS levels have been the focus of many studies (Sengel et al., 2019). In light of all this information, the alternative administration of a neuroprotective agent or its combinations would be an important development in terms of preventing secondary damage. Although VPA and its salts are antiepileptic agents with widespread effects, conflicting results have been obtained regarding their effects in modern animal experiments. The recent announcement that VPA has regulatory effects on histone deacetylases (HDAC), Gama aminobütirik asit (GABA), brain derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) is a positive development, contrary to the criticisms made (Thomas, 2014). For this purpose, we aimed to investigate whether VPA application has an effect against oxidative stress in affected spleen tissue caused by experimental SCI in rats.

Material and Methods

Sixteen male Wistar albino rats were purchased from the Animal Experiments department of the Veterinary Control Institute of Elazig. Rats with an average weight of 230 grams were randomly divided into two groups of an equal number. A clamp was applied to the bifurcation of the abdominal aorta and the infrarenal aorta for 45 minutes to create experimental SCI in each rat in all groups. No treatment was administered to the rats in Group 1 (SCI-(Control)), but a single dose of 300 mg/kg intraperitoneally VPA was administered to the rats in Group 2 (SCI-VPA). At the end of the 48-hour experimental period, all rats were decapitated under anesthesia, and spleen tissues were removed, washed in salt water, and frozen at -80°C until they were used for biochemical analysis. Subsequently, the frozen samples were weighed and homogenized, and the supernatants were obtained by subjecting the homogenate to centrifugation. In order to detect SOD activity in spleen tissue samples, Cayman's SOD assay kit was purchased, and related procedures were performed and for spleen tissue, it was expressed as U/mg protein. TOS and TAS levels were measured using commercial kits (Erel commercial kit was used) and the results were expressed as $\mu\text{molH}_2\text{O}_2\text{Eq/mg protein}$ and mmol Trolox Eq/mg

protein, respectively. This experimental study was conducted with the approval of the Local Ethics Committee for Animal Experiments of the Institute of Veterinary Control of Elazig (2022/04, EVKEM) and all protocols were applied.

Statistical Analyses

Statistical analyses were performed using the SPSS 20 version Windows package program. The results were given as mean, standard deviation. Kruskal-Wallis and Mann-Whitney tests were used as statistical methods between the groups, and the 'p' value of less than 0.05 was considered statistically significant

Results

Biochemical Assessment

The Table 1 shows the average TAS, TOS, and SOD levels of spleen tissue in the two groups. The TOS level, which is an important determinant of oxidative damage, was significantly lower in SCI-VPA compared to SCI-(Control). However, the TAS level and SOD activity were significantly higher ($P<0.05$). Thus, it is shown in the table that VPA regulates the antioxidant level, which reduces oxidative damage with its neuroprotective effect.

Table 1. Average standard values and p values of the groups compared to SCI-(Control).

	Median value	According to SCI-(Control) ($P<0.05$ value)
TOS ($\mu\text{molH}_2\text{O}_2\text{Eq/mg protein}$)		
SCI-(Control)	3.19	
SCI-VPA	2.70	0.0055
TAS ($\mu\text{molTroloxEq/mg protein}$)		
SCI-(Control)	4.18	
SCI-VPA	7.22	0.02
SOD (U/mg/protein)		
SCI-(Control)	4.20	
SCI-VPA	7.79	0.02

Discussion

SCI, which occurs for various reasons, including the creation of experimental SCI, is more dependent on the secondary injury when examined from the point of view of the organ it affects. Secondary injury is an event that occurs through the activation of many pathophysiological pathways that eventually lead to the death of the cell. One of these pathways is oxidative stress caused by anaerobic respiration, triggered by a lack of oxygen and energy due to ischemia. Oxidative stress shifts the body's oxidant/antioxidant balance in favor of oxidants and increases the production of ROS or reactive nitrogen species that damage cells. Constantly produced ROS can damage many structures, including the lipid contained in the cell structure, and therefore many organs can be affected by it (Luo et al., 2020). Several reports have shown that splenic damage occurs in rats exposed to ischemia reperfusion-induced oxidative stress. In order to effectively reduce organ damage caused by oxidative stress, intervention in free radical production with pharmacological agents should be considered (Sun et al., 2016). This study shows that VPA, which has many beneficial properties, including

neuroprotective, prevents spleen organ damage triggered by SCI. In addition, research on both effective SCI treatment and the antioxidant effect of VPA is quite limited. Therefore, these findings are promising in terms of reducing oxidative stress caused by SCI. TAS and TOS levels are commonly used markers to determine the total oxidative stress during SCI (Aras et al., 2015). Previous studies have reported that neurological damage and loss of function caused by ROS-induced oxidative stress is an important condition in SCI. This situation shows that ROS has critical roles in initiating mechanisms that mediate lipid peroxidation and oxidative damage after ischemia in various organ cells (Fatima et al., 2015). This study has shown that the TOS level decreases with the application of VPA in the spleen tissue during SCI, but the TAS level increase significantly. Enzymatic antioxidant enzymes are the first line of defense against oxidative stress, and SOD is one of the most important elements of this line (Villa et al., 2019). In this study, a significant decrease in SOD activity was observed in SCI-induced spleen tissue. However, the administration of VPA reduced SCI-induced oxidative stress in the spleen tissue by increasing SOD activity. In one study, it was shown that the administration

of VPA during acute SCI reduces motor neuron death by preventing both oxidative stress and endoplasmic reticulum stress in spinal tissue (Lee et al., 2012). Inhibition of previously increased MDA levels has been reported in rats treated with VPA following SCI. The neuroprotective effects of VPA, mediated by the upregulation of antioxidant enzyme levels, have previously been demonstrated under various oxidative stress conditions (Terzioğlu Bebitoğlu et al., 2012). These findings were consistent with a previous study that determined that the administration of VPA significantly maintained the level of antioxidant enzymes and thus prevented ischemia-reperfusion in Wistar rats (Zhang et al., 2012). In conclusion, the results of this study provided evidence that pharmacological intervention with VPA prevents oxidative stress in spleen tissue caused by sci and contributes to the level of antioxidant enzymes.

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