The effect of alpha-lipoic acid on nerve tissue healing after sciatic nerve crush injury in rats

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ARTICLE INFO

Keywords: Alpha-lipoic acid
Crush injury
Peripheral nerve
Rat
The sciatic nerve

Aim: Crush injury damages the nerve, affects its function and causes oxidative stress. Alpha-lipoic acid (ALA) is an antioxidant agent with protective effects on the nerve tissue. In this study, we aimed to investigate the effects of ALA in the treatment of crush sciatic nerve injury in rats.

Materials and Methods: Forty rats were divided into five groups. Walking Track Analysis (WTA) was performed in all groups before sacrificing the sciatic nerve. In group I (sham group), the sciatic nerve was exposed but not crushed, whereas in group II (early control group, 24th hour), group III (late control group, 7th day), group IV (early experimental group, 24th hour), and group V (late control group, 7th day), the sciatic nerve was exposed and clipped with an aneurysm clip for 300 seconds. One hour after the crush injury, subjects in groups II and III were given saline (2.5 ml, intraperitoneally), while ALA (100 mg/kg, intraperitoneally) was administered in Groups IV and V. WTA was performed in Groups I, II, and IV at the 24th hour after clipping and was performed in Groups III and V at 7th day after clipping. In all groups, the Sciatic Functional Index (SFI) was calculated after WTA. Following the completion of WTA, sciatic nerve tissue samples were obtained for the measurement of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) enzyme values.

Results: The SFI scores in groups II, III, IV, and V were significantly lower than that of sham group (p<0.05), while no significant difference was found between groups II and IV and between groups III and V (p>0.05). The CAT values of groups II and IV, the GSH-Px value and MDA value of group IV, and the SOD values of groups II and IV were found to be significantly higher than those of sham group (p<0.05). However, no significant difference was found among groups I, III, and V with regard to CAT, GSH-Px, MDA and SOD values (p>0.05).

Conclusion: The results indicated that a single dose of ALA (100 mg/kg) administered intraperitoneally one hour after the sciatic nerve crush injury had no therapeutic efficacy at 24 hours and 7 days after the administration. Further experimental studies are needed to evaluate the effectiveness of ALA applied in several doses rather than a single dose in crush peripheral nerve injury models.

Introduction

Etiological factors that cause peripheral nerve injuries (PNIs) are highly variable, and these injuries are mostly caused by trauma. The incidence of traumatic PNIs is 2-2.8% [1]. Blunt trauma has many types, including laceration, contusion, stretching, and traction, penetrating and perforating injuries, abnormal sleeping positions, external pressure, internal compression, ischemia and injection that may occur during anesthesia, electric shock, and thermal injury [2]. After PNI, ischemic and inflammatory processes begin, which may increase neurological symptoms. Experimental studies have tried various drugs, metabolites, and chemicals have been tried for PNI treatment and many studies are still ongoing [3].

Alpha-lipoic acid (ALA) is a naturally occurring com-
Groups

study.

ALA is an antioxidant that plays an important role as a cofactor in most mitochondrial reactions and with immunomodulatory and neuroprotective effects [5]. An important feature of ALA is its water and oil solubility and thus crosses the blood-brain barrier and is easily distributed throughout the body, including the nervous system [6].

ALA has significant antioxidant effects and a protective effect on nervous tissues in experimental models of central and peripheral nervous system disorders such as trauma, stroke, and diabetes, and has been recommended for treating oxidative disorders of the nervous system [7]. One of our previous studies revealed that the protective ALA application in a rat model before injury reduces nerve tissue damages in PNI [8]. Few studies on the therapeutic efficacy of post-injury administration of ALA in PNI are reported. In real life, patients present to hospitals after trauma, thus the protective effective of ALA does not matter in daily practice. We planned a study in the same experimental protocol in our previous study, in which we demonstrated the presence of a protective effect, to evaluate the therapeutic effect of post-traumatic ALA application.

Materials and Methods

In this study, as a hypothesis, we aimed to investigate the effects of ALA in the treatment of crushed sciatic nerve injury in rats. This study was conducted in Bağcılar Training and Research Hospital Experimental Research and Skills Development Center. Prior to the study, an approval was obtained from the Ministry of Health Istanbul Bağcılar Training and Research Hospital Animal Experiments Ethics Committee (Date: 02.07.2015; No: 2015/31). The study included 40 female Sprague-Dawley rats weighing 250-350 g. All the animals had free access to a standard diet and tap water.

Forty animals with the same characteristics were randomly assigned to each group (five groups) and then the experiments were started. Non-probable (haphazard sampling) sampling method and single-blind method was used in this study.

Groups

- I. Sham group: The sciatic nerve was explored and the incision was closed without performing any procedure. Sciatic nerve samples were taken 24 h later.
- II. Early control group: The sciatic nerve was explored, and 100 mg/kg intraperitoneal (IP) saline was administered 1 h after nerve crush injury induction. Sciatic nerve samples were taken 24 h after the nerve crush injury.
- III. Late control group: The sciatic nerve was explored and 100 mg/kg IP saline was administered 1 h after nerve crush injury induction. Sciatic nerve samples were taken 24 h after the nerve crush injury.
- IV. Early experimental group: The sciatic nerve was explored and 100 mg/kg IP ALA injection was administered 1 h after inducing nerve crush injury. Sciatic nerve samples were taken 24 h after the nerve crush injury.
- V. Late experimental group: The sciatic nerve was explored and 100 mg/kg IP ALA injection was administered 1 h after inducing nerve crush injury. Sciatic nerve samples were taken 7 days after the nerve crush injury.

Walking Track Analysis (WTA) was performed and the Sciatic Functional Index (SFI) was calculated for clinical evaluation in all groups before the experiment, at a time after inducing sciatic nerve crush injury and before collecting sciatic nerve samples. Sciatic nerve tissue samples obtained from all experimental groups were placed on saline-containing centrifuge tubes.

**Surgical technique**

The animals were anesthetized with 7 mg/kg of xylazine and 80 mg/kg of ketamine. Under a surgical microscope, the right sciatic nerve was explored by dissecting the biceps in the right gluteal region. Subsequently, compression was performed for 5 min using a titanium aneurysm clip at a location approximately 1.5 cm proximal to the separation point of the tibial and peroneal nerves to create crush injury on the nerve, and then a 5-min waiting period was employed for revascularization.

**WTA and SFI**

WTA and SFI were employed to evaluate the motor function of the rats. A walking track (length: 42 cm, width: 8.2 cm, and height: 12 cm), which had an open end to establish a path to a dark room, was used for evaluating the walking ability of the rats. A white-colored graph paper was placed on the walkway and then both hind paws of the rat were dipped in ink and the rat was required to walk along the walkway several times. After the rat learned to walk toward the darkroom, the most appropriate footprints on the paper were used for the analysis. In the footprints, the distance between the heel and the most distal part of the toe (print length [PL]), the distance between the first and fifth toes (toe spread [TS]), and the distance between the second and fourth toes (intermediate TS [ITS]) were measured using a millimeter ruler. The values obtained after measurements were used for calculating the SFI based on the formula developed by De Medinacelli and later modified by Bain et al. 

\[ SFI = \frac{38.3x[EPL-NPL]/NPL+109.5x[ETS-NTS]/NTS+13.3x[EIT-NIT]/NIT]-8.8 \quad \text{The resultant SFI value varied between 0 and -100, whereby “0” indicated normal function and “-100” indicated a complete loss of function. The SFI values were statistically compared among the groups [9,10].} \]

**Biochemical measurements**

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) measurements were made from the tissue samples obtained from the subjects. Measurements were performed using the Micro enzyme-linked immunosorbent assay (ELISA) method with a commercially available ELISA kit (Sunred Biotechnology Company).
Statistical analysis
In order to test the hypothesis of the research, the following statistical methods were used. Data were analyzed using the Statistical Package for the Social Sciences for Windows version 23 (Armonk, NY: IBM Corp.). Descriptive data were expressed as frequencies (n), percentages (%), mean, standard deviation, median, and minimum-maximum values. The normal distribution of variables was assessed using Kolmogorov-Smirnov test. Independent continuous variables were compared using the One-way analysis of variance followed by the posthoc Tukey test as well as the Independent Samples t-test, Kruskal-Wallis and Mann-Whitney U test. A p value of < 0.05 was considered significant.

Results
SFI
The SFI scores of the sham group were significantly higher than those of the early control and early experimental groups (p<0.05), whereas no significant difference was found between the early control and early experimental groups (p>0.05). The scores of the sham group were significantly higher than those of the late control and late experimental groups (p<0.05), whereas no significant difference was found between the late control and late experimental groups (p>0.05). Moreover, no significant difference was found between the scores of early and late control groups (p>0.05). Similarly, no significant difference was established between the early and late experimental groups (p>0.05). All the SFI scores are presented in Tables 1, 2, 3,4 and Figure 1.

Biochemical analysis
SOD determination
A significant difference was found among the sham, early control, and early experimental groups about SOD value (p<0.05). The mean SOD value (ng/mg tissue) was significantly lower in the sham group compared to the early control and experimental groups (p<0.05). No significant difference was found between the early control and early experimental groups, among the sham, early control, and early experimental groups, and between the early and late experimental groups (p>0.05). All the SFI scores are presented in Tables 1, 2, 3,4 and Figure 1.
Table 1. Early-term laboratory parameters (24 hours after clipping).

<table>
<thead>
<tr>
<th></th>
<th>Sham Group</th>
<th>Early control</th>
<th>Early Experimental</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT ng/mg tissue</td>
<td>0.696 ± 0.262</td>
<td>0.961 ± 0.193*</td>
<td>1.060</td>
<td>0.005</td>
</tr>
<tr>
<td>GSH-Px ng/mg tissue</td>
<td>0.533 ± 0.175</td>
<td>0.702 ± 0.114</td>
<td>0.811 ± 0.225*</td>
<td>0.0017</td>
</tr>
<tr>
<td>MDA nmol/mg tissue</td>
<td>0.159 ± 0.040</td>
<td>0.206 ± 0.052</td>
<td>0.240 ± 0.050*</td>
<td>0.010</td>
</tr>
<tr>
<td>SOD ng/mg tissue</td>
<td>0.283 ± 0.073</td>
<td>0.419 ± 0.056*</td>
<td>0.405 ± 0.089*</td>
<td>0.002</td>
</tr>
<tr>
<td>SFI</td>
<td>-8.45 ± 5.4</td>
<td>-58.9 ± 6.4*</td>
<td>-53.9 ± 10.2*</td>
<td>0.000</td>
</tr>
</tbody>
</table>

^ ANOVA (Tukey test) / ^ Kruskal-Wallis (Mann-Whitney U test. * Compared to sham group p<0.05. SD: Standard deviation, CAT: Catalase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, SOD: Superoxide dismutase, SFI: Sciatic Functional Index.

Control groups (p>0.05). The mean SOD value was significantly higher in the early experimental group compared to the late experimental group (p<0.05). SOD values are presented in Tables 1, 2, 3, 4 and Figure 2.

CAT determination

A significant difference was found among the sham, early control, and early experimental groups (p<0.05). The mean CAT value (ng/mg tissue) was significantly lower in the sham group compared to early control and early experimental groups (p<0.05), whereas no significant difference was found between the early control and early experimental groups and among the sham, late control, and late experimental groups (p>0.05). The mean CAT value (ng/mg tissue) was significantly higher in the early control group compared to the late control group and in the early experimental group compared to the late experimental group (Figure 2).

Table 2. Late-term laboratory parameters (7 days after clipping).

<table>
<thead>
<tr>
<th></th>
<th>Sham Group</th>
<th>Late control</th>
<th>Late Experimental</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT ng/mg tissue</td>
<td>0.696 ± 0.262</td>
<td>0.690 ± 0.215</td>
<td>0.659</td>
<td>0.945</td>
</tr>
<tr>
<td>GSH-Px ng/mg tissue</td>
<td>0.533 ± 0.175</td>
<td>0.609 ± 0.188</td>
<td>0.543</td>
<td>0.958</td>
</tr>
<tr>
<td>MDA nmol/mg tissue</td>
<td>0.159 ± 0.040</td>
<td>0.152 ± 0.028</td>
<td>0.157</td>
<td>0.911</td>
</tr>
<tr>
<td>SOD ng/mg tissue</td>
<td>0.283 ± 0.073</td>
<td>0.344 ± 0.053</td>
<td>0.323</td>
<td>0.395</td>
</tr>
<tr>
<td>SFI</td>
<td>-8.4 ± 5.4</td>
<td>-57.3 ± 4.6*</td>
<td>-61.8 ± 9.36*</td>
<td>0.000</td>
</tr>
</tbody>
</table>

^ ANOVA (Tukey test) / ^ Kruskal-Wallis (Mann-Whitney U test. * Compared to sham group p<0.05. SD: Standard deviation, CAT: Catalase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, SOD: Superoxide dismutase, SFI: Sciatic Functional Index.

Table 3. Early-term control and Late-term control laboratory parameters.

<table>
<thead>
<tr>
<th></th>
<th>Early Control</th>
<th>Late control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT ng/mg tissue</td>
<td>0.961 ± 0.193</td>
<td>0.690 ± 0.215</td>
<td>0.022</td>
</tr>
<tr>
<td>GSX ng/mg tissue</td>
<td>0.702 ± 0.114</td>
<td>0.673 ± 0.178</td>
<td>0.046</td>
</tr>
<tr>
<td>MDA nmol/mg tissue</td>
<td>0.206 ± 0.052</td>
<td>0.167 ± 0.048</td>
<td>0.134</td>
</tr>
<tr>
<td>SOD ng/mg tissue</td>
<td>0.419 ± 0.056</td>
<td>0.338 ± 0.119</td>
<td>0.105</td>
</tr>
<tr>
<td>SFI</td>
<td>-58.9 ± 6.4*</td>
<td>-57.3 ± 4.6</td>
<td>0.577</td>
</tr>
</tbody>
</table>

^ Independent Samples t-test / ^ Mann-Whitney U test.

Table 4. Early-term experimental and Late-term experimental laboratory parameters.

<table>
<thead>
<tr>
<th></th>
<th>Early Experimental</th>
<th>Late Experimental</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT ng/mg tissue</td>
<td>1.096 ± 0.189</td>
<td>0.662 ± 0.215</td>
<td>0.001</td>
</tr>
<tr>
<td>GSX ng/mg tissue</td>
<td>0.811 ± 0.225</td>
<td>0.560 ± 0.188</td>
<td>0.029</td>
</tr>
<tr>
<td>MDA nmol/mg tissue</td>
<td>0.240 ± 0.050</td>
<td>0.166 ± 0.028</td>
<td>0.003</td>
</tr>
<tr>
<td>SOD ng/mg tissue</td>
<td>0.405 ± 0.089</td>
<td>0.331 ± 0.053</td>
<td>0.046</td>
</tr>
<tr>
<td>SFI</td>
<td>-53.9 ± 10.2</td>
<td>-61.8 ± 9.36*</td>
<td>0.130</td>
</tr>
</tbody>
</table>

^ Independent Samples t-test / ^ Mann-Whitney U test.
mental group (p<0.05). CAT values are presented in Tables 1, 2, 3, 4 and Figure 3.

**GSH-Px determination**

A significant difference was found among the mean GSH-Px value (ng/mg tissue) of the sham, early control, and early experimental groups (p<0.05). The mean GSH-Px value was significantly higher in the early experimental group compared to that of the sham group (p<0.05), whereas no significant difference was found among the values of the other groups (p>0.05). Similarly, no significant difference was found among the mean values of the sham, late control, and late experimental groups (p>0.05). The mean GSH-Px value was significantly higher in the early control group compared to the late control group and in the early experimental group compared to the late experimental group (p<0.05). GSH-Px values are presented in Tables 1, 2, 3, 4 and Figure 4.

**MDA determination**

A significant difference was found among the mean MDA value (nmol/mg tissue) of the sham, early control, and early experimental groups (p<0.05). The mean MDA value was significantly lower in the sham group than that in the early experimental group (p<0.05), whereas no significant difference was found among the other groups (p>0.05). No significant difference was established among the mean MDA values (nmol/mg tissue) of the sham, late control, and late experimental groups and between the mean MDA value (nmol/mg tissue) of the early and late control groups (p>0.05). However, the mean MDA value (nmol/mg tissue) of the early experimental group was significantly higher than that of the late experimental group (p<0.05). MDA values are shown in Tables 1, 2, 3, 4 and Figure 5.

**Discussion**

PNI is highly common and causes severe disability due to motor and sensory dysfunctions. To date, various substances and drugs that are considered to reduce PNI have been tried to prevent the dysfunctions and are still being
ALA is a substance that has been tried in recent experimental studies in various PNIs and has been shown to exert protective activity [8,11]. Additionally, it has clinical applications for the treatment of peripheral nerve diseases caused by chronic compressions such as carpal tunnel syndrome and diabetic polyneuropathy [12,13]. Contrarily, experimental studies have shown that its effectiveness when applied before the injury as a preventative agent in acute PNI. However, most of the studies have evaluated its pre-traumatic protective effect and few studies evaluated its post-traumatic application that simulates real life. Therefore, the present study investigated the therapeutic efficacy of ALA.

Biological systems have special protective systems that can keep oxidative stress under control. The oxidative injury occurs in cases where the control mechanism remains insufficient and can be defined as the impaired of the balance between the ROS and antioxidant defense systems in favor of pro-oxidant substances and oxidant substances [18,19]. Antioxidants and ROS are constantly produced in living tissues. If antioxidants remain insufficient to neutralize oxidant substances, the balance is impaired in favor of oxidants, thereby causing oxidative stress. Toxic products, such as MDA, are formed due to the oxidation of cell membrane lipids by ROS. Excessively produced ROS are neutralized by antioxidants such as CAT, SOD, GSH-Px, glutathione reductase, endogenous glutathione (GSH), glutathione transferase (GST), and vitamins A, C, E to preserve tissue integrity and functions [20].

Measuring the level of ROS or antioxidants is frequently used to assess oxidative stress [21]. The isolated measurement of oxidants or antioxidants provides information about oxidative stress; however, the measurement of oxidants together with antioxidants provides greater information. Therefore, oxidant and antioxidant level should be simultaneously measured to better assess oxidative stress [22]. MDA is a product formed during lipid peroxidation and is frequently used in measurements due to its reaction with thiobarbituric acid [23].

Our study, measured the values of SOD, CAT, and GSH-Px, which play a role in the antioxidant system, and of MDA, which is a product of lipid peroxidation, in the injured nerve tissue. The main goal in nerve injury treatment is to restore impaired nerve function. Therefore, using functional studies as the evaluation criteria in studies that investigate the effects of different substances on nerve injuries is highly appropriate. Our study, evaluated
the functional recovery by performing pre- and post-injury WTA and calculating SFI, which was previously defined in rats [10].

Medications can also act as antioxidants in oxidative stages and reduce the harmful effects of the injury. Previous studies evaluated the antioxidant effects of various drugs and substances in different PNI models and investigated their protective function in the tissue against the adverse effects of the injury. Among these agents, ALA is an example of a drug with antioxidant effects, and many studies have shown that both lipoic acid and dihydrolipoic acid increase the antioxidant capacity of tissues against oxidative stress [24].

Many studies in the literature revealed the protective effect of ALA in PNI. Senoglu et al. applied ALA as a preventative in a peripheral nerve crush injury model and revealed that it provided increased tissue CAT and SOD levels and decreased in MDA levels after sciatic nerve injury. The authors also noted that the use of ALA before sciatic nerve injury showed a significant protective effect against nerve crush injury by reducing oxidative stress [25]. A study conducted in our clinic evaluated the effect of ALA before injury on the motor function and antioxidant enzyme activity in a peripheral nerve crush injury model and demonstrated the protective effect of ALA applied before injury both functionally and biochemically [8].

The only study in the literature that evaluated the therapeutic effect of ALA in crush PNI after trauma orally administered ALA and evaluated its effects using functional, molecular, and electron microscopy analysis. The results indicated that ALA achieved better recovery compared to other groups [26]. Some other studies evaluated the effectiveness of ALA applied after trauma in incision trauma models and showed the therapeutic efficacy of ALA [11,27].

In this study, which is a continuation of our previous study that demonstrated the protective efficacy of ALA before PNI, we evaluated the therapeutic effects of parenterally administered ALA after injury using the same study protocol. However, we observed that the parenteral administration of a single high dose of ALA after crush injury did not provide a significant improvement in the functional and biochemical parameters. This finding could be due to the limited application of ALA to a single dose. Therefore, further experimental studies are needed to evaluate the effectiveness of ALA in several doses rather than a single dose in crush PNI models.

Conclusion

Our study, evaluated the effects of a single dose ALA (100 mg/kg, i.p.) that is administered 1 hour after the induced nerve crush injury in the rat sciatic nerve on nerve healing. To achieve this, antioxidant enzyme values in the nerve tissue were measured and also functional evaluation was performed. The success of the crush injury model was demonstrated by the significant difference in both functional evaluation and antioxidant enzyme values in the experimental and control groups compared to the sham group. However, no significant difference was found between the experimental and control groups about functionality and antioxidant enzyme levels. Therefore, a single dose IP administered ALA (100 mg/kg) 1 hour after the sciatic nerve crush injury had no therapeutic efficacy. Accordingly, further experimental studies are needed to evaluate the effectiveness of ALA in several doses rather than a single dose in crush PNI models.

Disclosure

This study was presented at the Cervical and Lumbar Degenerative Spine Symposium held in Bodrum/Turkey on September 21-24, 2017 and was selected as the second-best study of the year.

Conflict of interest

Authors have no conflict of interest.

Ethical approval

Ministry of Health Istanbul Bağcılar Training and Research Hospital Animal Experiments Ethics Committee (Date: 02.07.2015; No: 2015/31).

References