The dose-dependent neuroprotective effect of alpha-lipoic acid in experimental spinal cord injury

Zale¿ne od dawki neuroprotekcyjne dzia³anie kwasu alfa-liponowego w eksperymentalnym uszkodzeniu rdzenia krêgowego

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Abstract

Background and purpose: Free radical production after spinal cord injury (SCI) plays an important role in secondary damage. The aim of this study was to investigate neuroprotective effects of the powerful antioxidant alpha-lipoic acid (ALA) in a spinal cord clip compression injury model.

Material and methods: Fifty-six Sprague-Dawley rats, weighing between 210 and 300 g, were randomly divided into seven groups. Spinal cord injury was performed by an aneurysm clip placed extradurally at the level of T9. Group 1 (sham) received laminectomy only. Group 2 (control) received SCI; Group 3 received 30 mg/kg of methylprednisolone sodium succinate (MPSS); Groups 4, 5, 6 and 7 received ALA at doses of 50, 100, 150, 200 mg/kg, respectively, via the intraperitoneal route immediately after SCI. The rats were neurologically tested 24 hours after trauma. Spinal cord samples from injury sites were harvested for measurement of lipid peroxidation products and histopathological evaluation.

Results: Spinal cord malonyldialdehyde levels of rats in treatment groups decreased after administration of ALA. The difference between the trauma group and groups receiving MPSS-ALA was statistically significant. The difference between the ALA (50, 100, 150 mg/kg) and MPSS groups was insignificant. Group 7 (ALA 200 mg/kg) was excluded.

Streszczenie

Wstêp i cel pracy: Powstawanie wolnych rodników po urazowo-wym uszkodzeniu rdzenia krêgowego (UURK) odgrywa istotn¹ rolê w jego wtórnym uszkodzeniu. Celem badania by³o sprawdzenie neuroprotekcyjnego dzia³ania silnego antyoksydantu, kwasu alfa-liponowego (ALA), w eksperymentalnym modelu uszkodzenia rdzenia krêgowego wywo³ywanego uci- skiem przez klips.

Materia³ i metody: Piêædziesi¹t szeœæ szczurów rasy Sprague-Dawley o masie 210–300 g podzielono na 7 grup. Uraz rdzenia krêgowego wywo³ywano przez za³o¿enie klipsa zewn¹trz-twardówkowo na poziomie Th9. W grupie 1. (procedura pozorowana) wykonano jedynie laminektomiê, w grupie 2. (kontrolnej) wykonano UURK. W grupie 3. podano bursztynian sodowy metyloprednisonolum (MPSS); Grupie 4., 5, 6 i 7 podano ALA w dawkach odpowiednio 50, 100, 150 i 200 mg/kg, odpowiednio, w kontrahonce otrzewno-po(20,133),(988,205)

Wyniki: Stê¿enia malonylodialdehydu u szczurów podda-nych leczeniu zmniejszy³y siê w grupach, w których stosowa-no ALA. Ró¿nica miêdzy grup¹ kontroln¹ a grupami otrzy-
from the study because of the possible toxic effect. Alpha lipoic acid and MPSS had similar effects on spinal cord injury in terms of lipid peroxidation, neurological recovery and histopathological changes.

**Conclusions:** Alpha lipoic acid at a dose range of 50-150 mg/kg is as effective as MPSS (30 mg/kg) in neuroprotection after SCI. Further, more detailed experimental studies are needed to determine the effects of ALA on the detrimental results of secondary SCI before its use in humans.

**Key words:** spinal cord injury, alpha-lipoic acid, free radical, lipid peroxidation, methylprednisolone.

**Introduction**

A lot of research has focused on pathophysiology of acute spinal cord injury (SCI) to find methods to restore neurological function. Acute injury involves two different, interrelated mechanisms of damage to the spinal cord: primary mechanical injury and a subsequent secondary injury because of additional damage after the initial injury [1]. The main autodestructive processes are secondary ischaemic changes, free radical formation, and free radical-induced lipid peroxidation with damage of lipid membranes in the spinal cord [2]. The exact mechanism is unknown, but many pathological changes, including edema, inflammation, altered blood flow, and changes in microvascular permeability, may contribute to the development of secondary injury by various pathways that include free oxygen radicals and lipid peroxidation [3-6]. Among these mechanisms, secondary injury draws much attention because of its nature, being susceptible to pharmacological intervention. Therefore, many studies have focused on neuroprotective agents against secondary injury [3-9].

Free radical production after SCI plays an important role in secondary injury [10-12]. Alpha-tocopherol, selenium, naloxone, and methylprednisolone sodium succinate (MPSS) are powerful antioxidants when given in large doses, and they effectively reduce posttraumatic neurological deficits [10-12]. According to a Cochrane Review [5], after spinal cord trauma 'high-dose methylprednisolone steroid therapy is the only pharmacological therapy shown to have efficacy when it can be administered within 8 hours of injury'. It is known that alpha-lipoic acid (ALA) is a powerful lipophilic antioxidant in vitro and in vivo [13]. Alpha-lipoic acid is an important co-factor for several mitochondrial dehydrogenases and, along with its reduced form, dihydrolipoic acid (DHLA), participates in redox reactions involved with acyl group transfers [14].

Despite recent improvements in surgical techniques, currently there is no efficacious therapy for SCI. An ideal treatment for secondary SCI would be one administered systemically without significant side effects. One currently untested therapeutic agent is ALA, which has been shown by some authors to possess potent neuroprotective properties in experimental cerebral ischaemia and reperfusion injury in peripheral nerves [14,15].

The aim of this study was to investigate neuroprotective effects of the powerful antioxidant ALA in a spinal cord clip compression injury model.

**Material and methods**

**Description of groups**

Fifty-six adult female Sprague-Dawley rats weighing 210-300 g were used for the study. Animals were housed under standard conditions in the Animal Research Laboratory at Celal Bayar University. Twelve-hour daylight and darkness cycles were normally applied before and after surgery. Room temperature was kept...
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The study protocol was approved by the Animal Research and Ethics Committee of Dokuz Eylul University (200906). The rats were given free access to water and food. The rats were randomly and blindly divided into seven groups, each containing eight rats. In the control group (group 1), total laminectomies were performed and non-traumatized spinal cord samples were obtained immediately. In the trauma group (group 2), total laminectomies were performed and spinal cord samples were removed at 24 hours after trauma. In the MPSS group (group 3), total laminectomies were performed, and animals were given a single dose of 30 mg/kg MPSS (Mustafa Nevzat, Istanbul, Turkey) intraperitoneally, immediately after trauma; spinal cord samples were obtained at 24 hours after trauma. In the ALA groups (groups 4, 5, 6 and 7), total laminectomies were performed; animals were given a single dose of 50, 100, 150 or 200 mg/kg ALA, respectively (Thioctacid 600 T Injectable, Meda Pharma, Germany) intraperitoneally immediately after trauma and spinal cord samples were obtained at 24 hours after trauma.

Surgical procedures

All rats were anesthetized via bolus intraperitoneal injection of sodium pentobarbital (20 mg/kg) (Bayer Turk Company, Istanbul, Turkey). Rats were positioned prone on the operating table. A midline dorsal incision was done with sterile technique. The laminae and transverse processes of T9-T12 were exposed by gentle blunt dissection of paravertebral muscles. A self-retaining microretractor was placed in the operation area, and laminectomy was performed at T8-10. Strict bleeding control was maintained by using bone wax and a bipolar coagulator. An aneurysm clip (Yasargil FE751, closing pressure 30 ± 2 g) was applied extradurally for one minute on the thoracic spinal cord. Intrapertoneal MPSS or ALA was injected according to the group. After careful removal of the aneurysm clip, paravertebral fascia and skin were sutured separately with silk stitches. Complete closure of the surgical wound was achieved. Complicated cases, such as dural tearing or inadvertent spinal injuries, were excluded from the study.

Sacrifice of animals and sample preparation

Rats were killed by overdose of pentobarbital (200 mg/kg) after 24 hours. Spinal cords at the injury site were excised for a length of 2 cm: 1 cm rostrally and 1 cm caudally to the injury site. Tissue samples were immediately stored in a –20°C freezer for assays of malondialdehyde (MDA).

Determination of lipid peroxidation in traumatized spinal cord tissue

The levels of lipid peroxides in traumatized spinal cord tissue were measured as thiobarbituric acid-reactive material and determined using the method of Mihara and Uchiyama [14]. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. The assay procedure for lipid peroxide in spinal cord tissue was set up as follows. Tissues were homogenized in 10 volumes (wt/vol) of cold 1.5% KCl; 0.5 mL of homogenate was mixed with 3 mL of 1% of H₃PO₄ and 1 mL of 0.6% thiobarbituric acid. The mixture was then heated in boiling water for 60 minutes. After cooling, the color was extracted into 4 mL n-butanol, and the absorbance was recorded at 535 and 520 nm. Using tetramethoxypropane as the standard, tissue lipid peroxide levels were calculated as nanomoles per gram of wet tissue.

Functional evaluation

Functional evaluation of the rats was made 24 hours after trauma. Evaluation of maximum slope at which rats maintained themselves for 5 seconds was performed by the inclined plane technique of Rivlin and Tator [16]. Assessments of hind limb function during open field walking were performed by using the Basso, Beattie, and Bresnahan (BBB) scoring system as described previously [17]. Briefly, rats were gently adapted to the open field. Once a rat walked continuously in the open field, two examiners conducted a 4-minute testing session using the BBB locomotor rating scale [17]. The BBB scale rates were based on different levels of movements of the hind limbs, with 21 points being the maximum score. This test mainly evaluates the movement of joints, weight support and plantar placement of the paw, weight-supported plantar steps, and fore limb–hind limb coordination. Average BBB scores of both legs were used. The evaluations were performed in a blinded fashion.

Light microscopic evaluation

Two millimeters of cross-sectional gray and white matter at the epicenter of the spinal cord lesion was removed for microscopic examination. The sections were
stained with hematoxylin-eosin (HE) and modified Gomori trichrome (MGT). The investigator evaluating the sections was blinded to the group information.

**Statistical analyses**

All data were obtained and originally analyzed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA), by researchers who were blinded to the treatments the rats had received. Comparison among groups was done with Kruskal-Wallis analysis of variance and the Mann-Whitney U-test. Results are expressed as mean ± standard deviation, median, and range. The differences were considered significant when p-values were less than 0.05.

**Results**

**Inclined plate tests and BBB scores**

Group 7 (ALA 200 mg/kg) was excluded from the study because of the possible toxic effect [18]. All rats in this group were dead at twelve hours after trauma and drug applications.

Inclined plane (IP) degree values showed statistically significant differences among groups. Trauma showed a statistically significant decrease in IP values (p < 0.05). Both MPSS and ALA groups were significantly different from the trauma group (p < 0.05). The difference between MPSS and ALA groups was not significant (p > 0.05). The comparison of ALA doses showed no significant difference (Table 1).

There was a statistically significant difference between the control and the trauma groups in the mean BBB scores (p < 0.05). Trauma was found to decrease BBB scores significantly (p < 0.05). The trauma group scores were significantly different from the MPSS or the ALA groups (p < 0.05). The comparison of ALA doses showed no significant difference (Table 2).

**MDA levels**

The MDA levels were highest in the trauma group and lower in the ALA and MPSS groups. Comparison of the MDA values of the groups with Kruskal-Wallis variance analysis revealed statistical significance (p < 0.05). When the groups were compared using post-hoc Mann-Whitney U-test, the results were as follows. When the trauma group was compared with the MPSS and ALA groups, the results were statistically significant (p < 0.05). The ALA treatment group demonstrated better results than the MPSS and the trauma groups numerically but showed no statistically significant difference between the MPSS group and the ALA group (p > 0.05). The comparison of ALA doses showed no significant difference (Table 3).

**Histopathological evaluation findings**

The control group demonstrated normal histological structures and it seems that trauma produced significant damage with prominent evidence of haemorrhage and cellular edema. The MPSS group showed...
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Fig. 1. Histological findings in trauma group included haemorrhage and necrosis.

Fig. 2. Histological findings in the group treated with methylprednisolone sodium succinate showed gliosis and loss of neurons.

Fig. 3. Histological findings in the group treated with alpha-lipoic acid showed gliosis and degenerated neurons.
evidence of haemorrhage with minimal cellular edema. In the ALA-treated groups (50, 100, and 150 mg/kg) histopathological structures were very similar and there was no significant difference from the MPSS group. Histopathological findings are presented in Figs. 1-3.

Discussion

Damage following SCI can be categorized as primary neuronal damage from the trauma itself and secondary damage caused by a cascade of events. Secondary spinal cord injury has been extensively studied in detail in recent decades. Secondary injury is considered as a result of biochemical and pathological interactions at the cellular level that causes increase in some excitotoxic neuromediators, excessive calcium release, reactive oxygen species (ROS) and lipid peroxidation and resulting edema, haemorrhage and ischaemia. All of them can cause dysfunction and death in neuronal cells [19]. ROS-induced lipid peroxidation is one of the most important components precipitating posttraumatic neuronal degeneration in the SCI [19]. Reactive oxygen species react with lipids and cause oxidative changes that result in elevated lipid peroxidation. The increase in lipid peroxidation may be related to decrease in enzymatic and nonenzymatic antioxidants of defense mechanisms. Due to high lipid content and high oxygenation, lipid peroxidation-related cellular damage might be easily formed by ROS [19].

Alpha-lipoic acid is especially promising as an antioxidant, acting against mitochondrial dysfunction, due to its mitochondrial alpha-ketoacid dehydrogenase complexes [13]. Thiols are central to antioxidant defense in nervous and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas ALA can. *In vitro*, animal and preliminary human studies indicate that ALA may be effective in preventing or treating numerous neurodegenerative disorders with this effect [13]. A review of current research reveals protective effects of ALA in cerebral ischaemia-reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue [13]. Demopoulos et al. [20] showed that the protective effect of methylprednisolone depends on its scavenging effects on free radicals more than its anti-inflammatory and immunosuppressive influences. Different studies showed that methylprednisolone has a strong limitation effect on lipid peroxidation, and it regulates microcirculation and cellular recovery with higher doses [9]. These results suggest that methylprednisolone may act through different mechanisms unrelated to corticosteroid receptors. The dose range of this beneficial effect is observed only at 30 mg/kg both in animal and clinical studies [21].

We aimed to investigate a currently untested therapeutic agent, the powerful antioxidant ALA, which has been shown by some authors to possess potent neuroprotective properties [14,15], in a spinal cord clip compression injury model.

The results of our study demonstrated that spinal cord MDA levels of rats in the treatment groups decreased after administration of ALA at a dose range of 50-150 mg/kg. The difference between the trauma and MPSS and ALA groups was statistically significant. The difference between the ALA doses and steroid groups was insignificant; this can be interpreted as ALA and MPSS having similar effects on spinal cord injury, in terms of lipid peroxidation, neurological recovery or histopathological tissue changes. Future studies, showing electron microscopic and immunohistochemical effects of ALA on spinal cord injury and lower doses (< 50 mg/kg) of ALA, should be planned to assess whether ALA also provides long-term neuroprotection after SCI.

Therefore, more and detailed experimental studies are needed to determine the effects of ALA on the detrimental results of secondary SCI, including lower dose applications.

Conclusions

Alpha-lipoic acid at a dose range of 50-150 mg/kg is as effective as MPSS (30 mg/kg) in neuroprotection after SCI.

Disclosure

Authors report no conflict of interest.

References