

Investigation of the Effects of Alpha Lipoic Acid Application on Total Antioxidant and Oxidant Status, Paraoxonase, and Total Sialic Acid Levels in Laminectomized Rabbits

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Summary

The objective of this study is to investigate the effect of intramuscular administration of alpha-lipoic acid (ALA) on total antioxidant (TAS), total oxidant (TOS), paraoxonase (PON) and total sialic acid (TSA) levels in laminectomized rabbits. In the study, twenty four white New Zealand rabbits weighing 3-4 kg were used. The investigation was carried out with three experiment and one control group consisting of six animals of each. No intervention has been done to the control group. Animals in the Group I received 50 mg/kg/day IM ALA (Thioctacid 600 T, M, EDA, Hamburg) without undergoing laminectomy. Group II underwent laminectomy; Group III underwent laminectomy and treated with IM ALA. The experiment lasted 45 days and blood samples were taken on 0, 15th, 30th, and 45th days from the animals. TAS, TOS, PON and TSA levels were measured in the serum. We conclude that ALA treatment would be beneficial in laminectomized rabbits in order to suppress inflammatory process and to expedite recovery.

Keywords: ALA, Laminectomy, TAS, TOS, PON, TSA, Rabbit

Laminektomi Yapılan Tavşanlarda Alfa-lipoik Asit Uygulamalarının Total Antioksidan Kapasite, Total Oksidan Kapasite, Paraoksonaz ve Total Sialik Asit Düzeyleri Üzerine Etkilerinin Araştırılması

Özet

Bu çalışma, laminektomi yapılan tavşanlarda intramüsküler Alfa Lipoik Asit (ALA) uygulamasının total antioksidan kapasite (TAS), total oksidan kapasite (TOS), paraoksonaz (PON), ve total sialik asit (TSA) düzeylerini üzerine olan etkilerini araştırmak amacı ile yapıldı. Çalışmada, 3.5-4 kg ağırlığında 24 adet beyaz Yeni Zelanda erkek tavşan kullanıldı. Araştırma, herbiri 6 adet hayvan içeren 1 kontrol ve 3 deneme grubu olmak üzere 4 grup halinde yürütüldü. Kontrol grubuna hiçbir müdahale yapılmadı. Grup I'deki hayvanlara laminektomi yapılmaksızın 50 mg/kg/gün İM ALA (Thioctacid 600 T, M EDA, Hamburg, Germany) enjekte edildi. Grup II'deki hayvanlara sadece laminektomi yapıldı. Grup III'e İM ALA+laminektomi uygulandı. Deneme toplamda 45 gün sürdürüldü. Denemenin 0., 15., 30.,ve 45. günlerinde vena auricularisten kan alındı. Elde edilen serum örneklerinde TAS, TOS, PON ve TSA düzeyleri belirlendi. Sonuç olarak, laminektomi yapılan tavşanlarda yangısal sürecin baskılanması ve iyileşmenin hızlandırılması için eksojen ALA kullanımının faydalı olabileceği kanısına varıldı.

Anahtar sözcükler: ALA, Laminektomi, TAS, TOS, PON, TSA, Tavşan



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INTRODUCTION

Alpha lipoic acid (ALA) has been described as a potent biologic antioxidant, a detoxifying agent, and a medicine for diabetes. It is used for the treatment of age-related cardiovascular and neuromuscular insufficiencies, and plays an important role as a modulator in various inflammatory signal pathways. Effect of ALA on inflammation originated from cytokines have been investigated and has been discovered that it was an inhibitor of NF-kappa β [1], matrix metalloproteinase-13 (MMP-13) and growth factor-p (TGF J-3) [1]. It has been reported that antioxidant property of ALA, which inhibits the liver fibrosis, arised from its natural tiol-antioxidant peculiarity [2]. Furthermore, ALA and its reduced form, dihidrolipoic acid (DHLA), play roles in the brain and other tissues as antioxidant defenders [3]. Anti-inflammatory properties of ALA have rarely been investigated in humans until today. It has been reported that ALA administration of 50 mg/day for 4 weeks results in 15% of reduction in Interleukin-6 (IL-6) levels which is an important indicator of inflammation, as well as an organizer of the expression of inflammatory cytokines as Interleukin 1 (IL-1) and Tumor Necrosis Factor α (TNF- α) [2].

Generally, oxidative stress is related to effect of increased free radical molecules resulting from metabolic disorders and other illnesses [4-6]. It has also been reported that free radicals were continuously produced in metabolic settings, and their levels significantly increase even in the physiologic circumstances [4]. It has been reported that some health problems occurred when the equilibrium was impaired between the oxidant and antioxidant substances [5-7]. In order to evaluate oxidative stress, although analyses of antioxidant parameters can be performed for this purpose, new methods like TAS have recently been developed [8,9]. To determine the oxidative index (OSI), ratio of the total peroxide value to total antioxidant capacity is calculated¹ It has been reported that only the TAS measurement can be used to determine the balance between the pro-oxidant and oxidant substances [6,10,11]. When poliansaturated fatty acids were oxidized with free radicals, lipid peroxide radicals emerge via LOOH producing chain reactions concomitant with the oxidation [12].

Reactive oxygen species and nitrogen-bearing free radicals (ROS and RNS) are being produced continuously during the aerobic metabolism and their levels increase significantly in the pathologic conditions [13,14]. Increase in the free radical levels also cause increases in the catabolic reactions in cells for the protection of homeostasis in the organisms [15]. Increased free radical levels cause cell and tissue destructions in the organism and elicits a status called "oxidative stress"[4,14,15].

Sialic acid (SA) is a derivative of neuroaminic acid [16,17] and abundantly present in all cell membranes [17-20]. It

has been reported that SA concentration had increased rapidly in the pathologic conditions like inflammation, tissue destruction, and tissue proliferation [17]. Due to this fact, evaluation of SA concentration may be an important sign in the diagnosis of inflammatory diseases [19,21-24]. Although the mechanism in the increase of serum SA level during the inflammatory conditions is not clear, many investigators indicated the role of AFPs which share the same structure with SA [17,19,20,25]. Since acute phase reactants are glycoprotein in structure, it has been reported that increase in these proteins affects the TSA levels [20] but not reflects the increases in SA levels exactly, which also bears glycoprotein structure [19].

Paraoxonase (PON1) is a member of the protein family. PON1 was primarily synthesized in liver and some of it, together with high density lipoproteins, was secreted into the plasma. PON1, beside the posphorilated insecticides (e.g chlorpirifos, oxon, diaxozon) and active metabolites hydrolyses nervous agents like sarin, soman, and VX [26,27]. Due to its antioxidant capacity, plays a protective role in phosphate intoxications and exerts a modulating effect in cardiovascular diseases.

One of the physiologic effects of PON1 is catching the low density lipoprotein particles (LDL) as well as the oxidized metabolized lipid byproducts. PON1 prevents the phospholipids included in HDL from oxidation as well [26-28].

The objective of this study is to investigate the effects of intramuscular ALA administration on TAS, TOS, PON and TSA levels in laminectomized rabbits.

MATERIAL and METHODS

Experimental Design

Twenty four adult New Zealand white male rabbits weighting 3.5-4 kg were used in this study. The study was accomplished at the Kafkas University, Local Ethical Committee of Animal experiments with approval (KAU-HADYEK 2012/85). All animals received humane care as outlined in the "Guide for the care and use of laboratory animals" [29]. The animals were deprived of food for 24 h before surgery, but were allowed free intake of water. The investigation was carried out with three experiment and one control group consisting of six animals of each. The animals in Group I received 50 mg/kg/day IM ALA (Thioctacid® 600 T, M, EDA, Hamburg, GERMANY) without undergoing laminectomy. Group II underwent laminectomy without treatment; Group III underwent laminectomy and was applied 50 mg/kg/day IM ALA. The duration of the experiment was 45 days and blood samples were taken on 0, 15th, 30th, and 45th days from the animals. TAS, TOS, PON and TSA levels were measured in the serum. Preoperative and postoperative analgesic and antibiotic prophylaxis was given for three groups of animals.

Surgery

Animals were anesthetized with intramuscular 9 mg/kg xylazine HCl (Rompun® Bayer, Istanbul, Turkey) and 60 mg/kg ketamine HCl (Ketalor®. Pfizer. Istanbul, Turkey) at 1/0.5 proportion, 0.1 mL/100 g body weight. Additional dose was applied for extending the anesthesia time if necessary; that was the 20% of the original dose of above mentioned medications. Following anesthesia, animals were stabilized on the operation table in prone position. The lumbar region was shaved and cleaned with the antiseptic providon iodine. The rectal temperature was recorded continuously and tried to be kept around 37°C during the whole surgical procedure. Following a 4-cm midline skin incision starting from the L5 level, the lumbar fascia was opened bilaterally from midline and bilateral subperiosteal dissection of paravertebral muscles was carried out.

The L5 level was determined by palpation of the iliac wings. L3 and L4 total laminectomy was carried out with a 1-mm Kerrison rongeur under an operating microscope at 10x magnification (Möller-Wedel®, Wedel. Germany), following the removal of spinous processes. The ligamentum flavum and epidural fat tissue were removed and the dura mater exposed. Following hemostasis, the operation field was irrigated and cleaned with physiological saline solution. Then after, operation was done.

Collecting the Blood Samples

For the biochemical analyses, blood sample were taken from the vena auricularis from all groups included in the study prior to application (0); and on 15th, 30th, and 45th days after the application. Blood samples were centrifuged at 3.000 rpm, serum samples were harvested, and stored in the -25°C freezer until they were analyzed.

Biochemical Analyses

Total sialic acid analyses (TSA): Serum TSA analyses were performed spectrophotometrically [30].

Measurement of the Total Antioxidant Status: Serum TAS level was determined spectrophotometrically on an auto-analyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits®, Gaziantep, Turkey) as described by Erel [31]. The assay had excellent precision values lower than 3% and results were expressed as mmol Trolox Eq/L.

Measurement of Total Oxidant Status: Serum TOS level of was measured spectrophotometrically on an auto-analyzer (Aeroset®, Abbott®, Illinois, USA) using commercial kits (Rel assay diagnostic kits®, Gaziantep, Turkey) as described by Erel [32]. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$).

Oxidative Stress Index: Percentage ratio of TOS to TAC level was assessed as OSI. For calculation, the resulting unit of TAC was converted to mmol/L, and the OSI value was calculated according to the following formula; $\text{OSI (Arbitrary Unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}) / \text{TAC mmol Trolox Eq/L}$ [32].

Measurement of Paraoxonase and Arylesterase Activity: Paraoxonase activity was measured in the absence (basal-activity) and presence of NaCl (salt-stimulated activity), using paraoxon substrate. The rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm. Paraoxonase activity was expressed as U/L serum [33,34].

Statistics

It was subjected to normality. Normality results were analyzed by nonparametric method and then multiple groups were examined by kruskal wallis method. After these analysis, dual comparison were done by Mann-Whitney U method. Analyses were performed with SPSS for Windows (version 20.0; SPSS, Chicago, IL) in a PC.

RESULTS

TAS, TOS, PON, TSA OSI and Paraoxonase levels obtained from this study are shown in [Table 1](#).

In the group II, significant increases were obtained on TSA, TOS and OSI levels in all treatment dates comparing with the values obtained before the operation ($P < 0.001$), but significant decreases on TAS and PON levels were seen ($P < 0.001$). It was determined statistically an important decrease in levels of TAS and PON activity as parallel to increase of the levels of OSI, TOS and TSA especially in the Group II compared to control Group from 15th day to finish of applications. It was determined statistically an important increase in levels of TAS and PON activity as parallel to decrease of the levels of OSI, TOS and TSA especially in the Group III compared to Group II from 15th day to finish of applications.

In the animals consisting the only Group I, values obtained from the samples taken from all treatment dates revealed nonsignificant decreases on TSA and TOS levels; but contrarily, significant increases on TAS and TOS values ($P < 0.001$).

DISCUSSION

In this study, ALA, which its anti-inflammatory specifications has rarely been searched until today, has been administered to laminectomized rabbits via intramuscular course and effects of the substance on total antioxidant capacity (TAS), total oxidant capacity (TOS), paraoxonase (PON), and total sialic acid levels

Table 1. TAS, TOS, OSI, PON and TSA values obtained from the study (mean±std.deviation)**Tablo 1.** Çalışmada elde edilen TAS, TOS, OSI, PON ve TSA değerleri (ortalama±std.deviasyon)

Parameters	Groups	Days				P
		0	15	30	45	
TAS (mmol Trolox Eq/L)	Control (n=6)	0.652±0.028	0.645±0.016 B	0.648±0.034 B	0.646±0.048 B	-
	Group I (n=6)	0.645±0.016 B	0.715±0.034 A.a	0.726±0.067 A.a	0.737±0.042 A.a	P<0.001
	Group II (n=6)	0.648±0.034 A	0.515±0.075 B.c	0.504±0.074 B.c	0.540±0.038 B.c	P<0.001
	Group III (n=6)	0.653±0.029 A	0.601±0.040 B.b	0.612±0.021 B.b	0.627±0.021 AB.b	P<0.008
	P	-	P<0.001	P<0.001	P<0.001	-
TOS (µmol H ₂ O ₂ Eq/L)	control (n=6)	0.552±0.028	0.545±0.016 C	0.548±0.034 Bc	0.546±0.048 C	-
	Group I (n=6)	0.545±0.016	0.530±0.029 C	0.533±0.021 C	0.531±0.024 C	-
	Group II (n=6)	0.543±0.026 B	0.677±0.021 A.a	0.650±0.024 A.a	0.656±0.033 A.a	P<0.001
	Group III (n=6)	0.541±0.022 A	0.595±0.035 B.b	0.571±0.013 B.b	0.581±0.012 B.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
OSI	Control (n=6)	0.846±0.006	0.844±0.003 C	0.845±0.007 Bc	0.844±0.010 B	-
	Group I (n=6)	0.844±0.003 A	0.741±0.037 AB.c	0.738±0.050 AB.c	0.632±0.259 B.c	P<0.05
	Group II (n=6)	0.838±0.015 B	1.341±0.278 A.a	1.316±0.263 A.a	1.219±0.092 A.a	P<0.001
	Group III (n=6)	0.829±0.045 C	0.990±0.025 A.b	0.933±0.043 B.b	0.927±0.0467 B.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
PON (U/L)	Control (n=6)	210.1±21.9	208.9±13.7 B	210.2±14.6 B	210.4±19.8 B	-
	Group I (n=6)	210.0±10.3 B	236.8±7.7 A.a	236.7±2.5 A.a	235.5±4.4 A.a	P<0.001
	Group II (n=6)	210.8±15.9 A	160.5±9.6 C.d	163.6±6.2 BC.d	172.1±6.4 B.c	P<0.001
	Group III (n=6)	209.2±12.0 A	184.4±5.6 B.c	190.2±7.7 B.c	201.5±7.8 A.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-
TSA (mg/dl)	Control (n=6)	60.25±2.12	60.50±1.69 C	59.50±1.51 C	59.37±1.40 C	-
	Group I (n=6)	60.50±2.00	58.75±1.90 C	58.62±2.06 C	58.12±1.24 C	-
	Group II (n=6)	60.12±5.11 D	89.12±2.85 A.a	84.12±3.27 B.a	80.25±2.96 C.a	P<0.001
	Group III (n=6)	60.25±2.60 D	78.12±3.22 A.b	74.12±2.58 B.b	71.00±2.72 C.b	P<0.001
	P	-	P<0.001	P<0.001	P<0.001	-

ABCD: Statistical significance of days in the groups; a.b.c.d: Statistical significance between the groups

have been investigated. Intramuscular ALA application also exerted positive effect on TSA, TAS, TOS and PON values.

Analysis of sialic acid provides important clues for the diagnosis and estimation of the prognosis of the diseases, and is being used increasingly nova days [17,25]. In our study show that, plasma SA levels of laminectomized animals were higher than the levels of laminectomy + ALA administered, and only ALA administered animal groups. High levels of plasma SA values in laminectomized animals may be considered as the result of more pronounced tissue damage in this group comparing with the Group III. It has been reported that SA concentration rapidly increases during the pathological circumstances since tissue destruction and proliferation occur [17]. We propose that, in accordance with the reports published by other investigators previously [18], these changes in SA levels originate from the over secretion of the lipid-bounded sialic acid compounds exerted by the sialoprotein synthesis

in the liver [18] and increased sialidase enzyme activity [35] during the inflammation [36].

After the ALA treatment, low levels of TSA and TOS values were obtained due to prevention of inflammatory effect of laminectomy, especially after 15th day of administration. In this experiment, it has been detected that inflammation and stress caused by laminectomy operation had induced increases in TOS levels. In a study conducted by Gutteridge and Halliwell [37], authors proposed that lipid peroxidation levels might have been provoked to rise by a defect in the antioxidant defense mechanism. We consider that high TOS and low TSA values were the consequences of tissue damage and lipidperoxidation in laminectomized animals arising from the operation. This finding is parallel to the data from a previous study reporting the preventive effect of ALA against tissue damage. It has been detected that Groups III resulted in small increase on TOS (P<0.001), but significant increase on TSA values only in the laminectomized group comparing with other groups. We share

the same opinion with others [2,3] that this outcome resulted from an effect exerted by ALA hindering the production of the reactive oxygen species (ROS) and other free radicals. It has been reported that ALA could be used against the destructive effect of ROS, and this application evoked increased TAS levels [2,3].

While statistically significant decreases in PON values were obtained in laminectomized group comparing with the other three (control, Group I, Group III), significant increase was observed in PON values in the group treated only by ALA ($P < 0.001$). In the Group III, PON values obtained on 15th, 30th, and 45th days of treatment showed significant decreases in comparison with the values obtained prior to operation ($P < 0.001$). We share the opinion with the others that decreased values of PON in Group I might have been the consequence of the close relation between the free sulphhydryl groups and the antioxidant capacity, and the antioxidant effect during the conditions of inflammation and stress [38,39]. PON1 which has paraoxonase, arylesterase and lactonase activities, is closely associated with high density lipoproteins (HDL)₁ and catalyzes the hydrolysis of a variety of aromatic carboxylic acid esters and several organophosphates and also a variety of lactones and cyclic carbonate esters, including naturally occurring lactones and pharmacological agents [40]. Paraoxonase activity which has decreased in oxidative stress conditions and belong to PON1 is used as an indicator in both toxicological studies and clinical cases [38-43]. In the present study, it was determined statistically an important decrease in PON1 activity as parallel to OSI levels especially in the Group II from 15th day to finish of applications.

The measurement of TAS and TOS using novel automated systems is likely to show the systemic production of all free radicals [31,32]. It has not run accrossed to the applied alpha lipoic acid on laminectomy. In this study were evaluated effect of alpha lipoic acid on TAS and TOS levels in laminectomy application. Additionally, the present study was not designed to analyze the possible discriminatory capacity of TOS/TAS (OSI) for laminectomy in the general population. The clinical value of the associations observed in our study will be analyzed as detail in another study.

As a result, in this study it has been proposed that laminectomy had caused alterations on TSA, TAS, TOS, and PON levels; on the other hand, administration of ALA had supported the antioxidant capacity of the plasma by increasing TAS and PON levels, but decreasing TSA and TOS levels in animals. We conclude that, in order to suppress the inflammatory process and to accelerate the healing, IM ALA treatment proves a promising method.

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