ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

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Investigation of the effects of lipoic acid and dihydrolipoate on experimental renal ischemia-reperfusion model

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Abstract

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Objective: Ischemic/reperfusion (I/R) causes tissue injury and the leading cause of acute kidney injury. In this study, we aimed to investigate the effects of the long and short-term usage of ALA and short-term DHLA on oxidative stress markers in the experimental renal ischemia-reperfusion model.
Method: Forty male rats (250 to 300 gr) were divided into 5 groups: control; I/R group; long-term ALA+IR group; short-term ALA+IR group; and short-term DHLA+IR group. Ischemia was carried out for 45 minutes followed by reperfusion for 4 hours. Thiobarbituric acid reactive sunstances (TBARM), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities in tissue samples and serum total antioxidant status (TAS) and total oxidative stress (TOS) assayed by the spectrophotometrically. Tissue samples were investigated by histopathological analyzes.
Results: TBARM (Control: 0.38±0.05. I/R: 1.37±0.17, long-term ALA-treated group:1.025±0.15, short-term ALA-treated group: 0.68±0.09, short-term DHLA-treated group: 0.38±0.04 (nmol/mg protein); p<0,001) CAT (Control: 0.12±0.02, I/R: 0.04±0.008, long-term ALA-treated group: 0.07±0.01, short-term ALA-treated group:0.06±0.008, short-term DHLA-treated group: 0.08±0.01 (k/mg protein); p<0.001), GSH-Px (Control: 0.45±0.04, I/R: 0.21±0.028, long-term ALA-treated group: 0.37±0.05, short-term ALA-treated group :0.34±0.05, short-term DHLA-treated group: 0.37±0.04 (U/mg protein); p<0.001), and serum OSI levels (Control: 1.32±0.15, I/R: 3.08±0.44, long-term ALA-treated group: 1.775±0.21, short-term ALA-treated group: 1.85±0.37, short-term DHLA-treated group: 1.53±0.21 (arbitrary unit) ; p<0.001) were improved in the long and short-term ALA-treated group and short-term DHLA-treated group compared to the I/R group. These findings were more prominent in histopathological tissue samples in the DHLA-treated group.
Conclusion: We consider that both long-term and short-term ALA applications have the potential for the treatment of renal I/R damage. Besides, DHLA is more effective than ALA.
Keywords: Renal Damage, Ischemia/Reperfusion, Lipoic Acid, Dihydrolipoate, Antioxidant Enzyme

Öz

Deneysel böbrek iskemi-reperfüzyon modelinde lipoik asit ve dihidrolipoat kullanımının etkilerinin incelenmesi

Amaç: İskemi/reperfüzyon (I/R) doku hasarına neden olarak akut böbrek hasarına yol açar. Bu çalışmada, deneysel böbrek iskemi-reperfüzyon modelinde uzun ve kısa süreli ALA ve kısa süreli DHLA kullanımının oksidatif stres belirteçleri üzerine etkilerinin araştırması amaçlanmıştır. .
Yöntem: Kırk adet erkek rat (250-300 gr) 5 gruba ayrılmıştır: kontrol grubu; I/R grubu; uzun vadeli ALA+IR grubu; kısa vadeli ALA+IR grubu ve kısa vadeli DHLA+IR grubu. 45 dakika süreyle iskemi, ardından 4 saat süreyle reperfüzyon uygulanmıştır. Doku örneklerinde Tiyobarbitürik asid reaktif maddeler (TBARM), katalaz (CAT), süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) aktiviteleri ile total antioksidan durum (TAS) ve total oksidatif stres kapasitesi (TOS) spektrofotometrik olarak ölçülmüştür. Doku örnekleri ayrıca histopatolojik olarak analiz edilmiştir.
Bulgular: Uzun ve kısa süreli ALA uygulanan grupta ve kısa süreli DHLA uygulanan grupta I/R grubuna kıyasla TBARM (Kontrol: 0.38±0.05, I/R: 1.37±0.17, uzun vadeli ALA+IR grubu: 1.025±0.15, kısa vadeli ALA+IR grubu: 0.68±0.09, kısa vadeli DHLA+IR grubu: 0,38±0,04 (nmol/mg protein); p<0.001), CAT (Kontrol: 0.12±0.02, I/R: 0.04±0.008, uzun vadeli ALA+IR grubu: 0,07±0,01, kısa vadeli ALA+IR grubu: 0,06±0,008, kısa vadeli DHLA+IR grubu: 0.08±0.01 (k/mg protein; p<0.001), GSH-Px (Kontrol: 0.45±0.04, I/R: 0.21±0.028, uzun vadeli ALA+IR grubu: 0.37±0.05, kısa vadeli ALA+IR grubu: 0.34±0.05, kısa vadeli DHLA+IR grubu: 0.37±0.04 (U/mg protein); p<0.001), ve serum OSI (Kontrol: 1.32±0.15, I/R: 3.08±0.44, uzun vadeli ALA+IR grubu: 1.775±0.21, kısa vadeli ALA+IR grubu: 1.85±0.37, kısa vadeli DHLA+IR grubu: 1.53±0.21 (arbitary unit); p<0.001) seviyelerinde iyileşme gözlemlenmiştir. Bu bulgular, histopatolojik doku örneklerinde DHLA uygulanan grupta daha belirgindir
Sonuç: Hem uzun süreli hem de kısa süreli ALA uygulamalarının renal I/R hasarının tedavisi için potansiyele sahip olduğu düşünülmektedir. Ayrıca DHLA'nın, ALA'dan daha etkili olduğu düşünülmektedir.
Anahtar Kelimeler: Böbrek Hasarı, İskemi/Reperfüzyon, Lipoik Asit, Dihidrolipoat, Antioksidan Enzim

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INTRODUCTION

Ischemic/reperfusion (I/R) causes tissue injury and currently the leading cause of acute kidney injury (AKI) in hospitalized patients undergoing surgical procedures including cardiopulmonary bypass, urological surgery or organ transplantation and systemic conditions such as sepsis and shock (1,2). Initial restriction of blood supply to renal tissue leads to an imbalance in metabolic supply and demand, causing hypoxia and hypoxia-induced reactive oxygen species (ROS) accumulation (3). Increased amounts of ROS within the cell and insufficiency of antioxidant levels may lead to deterioration of cell membranes, intracellular proteins, and DNA structure (4). Subsequent reperfusion and concomitant reoxygenation further enhances tissue injury by increasing ROS production and inflammatory activation. At the same time, it has been known that ROS are powerful mediators of renal endothelial and tubular cell injury (5).

Various antioxidants are used to be potential candidates for the prevention or treatment of I/R-induced renal injury (6,7). α-Lipoic acid (ALA) is a well-known antioxidant and has been shown to prevent I/R damage in many diseases including such as myocardial infarction, stroke, and peripheral vascular disease (8). These effects have attributed to its radical scavenging and metal chelation properties (9,10). There are two different forms of lipoic acid which can be converted to each other by oxidation-reduction reactions in tissues. Oxidized lipoic acid: sulphur based in positions 6 and 8 form, a closed ring structure. Reduced lipoic acid: Named as Dihydrolipoic acid (DHLA) is called, the sulphurs in the 6th and 8th positions are in the form of the sulfhydryl group (-SH). DHLA has a form of open chain structure (11). Both forms have biological activity and involved in the removal of ROS, chelation of metal ions, and repair of oxidatively damaged proteins (12). However, DHLA is more active than ALA because ALA mainly chelation of Fe^{+2} and Cu^{+2}, DHLA additionally chelation of Cd^{+2} and also plays role of regeneration of endogen antioxidants like vitamin C and E. (13). The effects of ALA on renal ischemia-reperfusion injury have been investigated in several studies, but there is no study investigating the effects of DHLA on renal damage.

In this study, we aimed to investigate the effects of ALA and DHLA on oxidative balance and compare their short and long-term effects in the experimental renal I/R model.

METHOD

Experimental Design

After the approval of the Ethics committee (2015/8-2), five groups were formed with 8 male mice of Wistar albino genus, 12-16 weeks old, weighting 300 to 350 gr in each. The groups were fed by normal ad libitum during 14 days.

Group I (control); Group II (I/R), ischemia/reperfusion was performed; Group III (long-term ALA+I/R) 100 mg lipoic acid was applicated intraperitoneally during 14 days and then ischemia reperfusion was performed; Group IV (short term ALA+I/R), 100 mg/kg lipoic acid was applied intraperitoneally as single dose just 2 hours before ischemia/reperfusion, Group V (short term DHLA+I/R), 100 mg/kg dihydrolipoat was applied intraperitoneally as single dose just 2 hours before ischemia/reperfusion. Unlike ALA, DHLA is a direct-acting biological agent, so has been examined only its short-term effects. The groups other than controls were anesthetized by giving 80 mg/kg ketamine and 12 mg/kg Xylazin. After opening from the midline of the abdomen; the left kidney artery was found and clamped with a bulldog clamp (Vascustatts, Scanlan, USA). After 45 minutes of ischemia, the clamps were removed and arteriovenous flow started again with the opening of the kidney colour and pulsation. At the end of 4 hours reperfusion, renal tissues were taken for biochemical and histopathological examination by nephrectomy, and blood samples were taken for biochemical analyses by intracardiac puncture, the experiment was terminated with sacrifice.

Collecting tissue and blood samples

Blood samples were taken into biochemistry tubes (BD, Blood Collection Tubes) and centrifuged at 1500 g for 10 min. Serum samples were taken and stored at -80 C until biochemical analysis.

Renal tissue was divided into two portion after removal, half was placed in 10% zinc formaldehyde for histopathological examination, and the other half was stored at -80 C to analyze the parameters for oxidative stress.

Biochemical measurements in tissue samples

Tissue samples were homogenized (IKA T 10 basic ULTRA-TURRAX Homogenizator) in 50 mM ice-cold phosphate buffer (pH: 7.00), and the supernatants were obtained by centrifugation of the homogenates at 15000 x g for 45 minutes.

Protein quantity was determined by Bradford method using Commasie Brillant Blue G-250 (14).

TBARM levels were measured by the double boiling method, modified by Hammouda et al. (15). Lipid peroxidation end products reacted with thiobarbituric acid were measured spectrophotometrically (Shimadzu™ UV 1800 Spectrophotometer) at 532 nm wavelength. The calibration curve was generated using commercially available MDA equivalents (1.1.3.3-tetramethoxypropane, Sigma-Aldrich).

Catalase activity was measured by the method of Aebi (1974); Hydrogen peroxide (H_2O_2) gives the maximum absorbance at 240 nm. H_2O_2, which is added to the

experimental environment, is broken down by catalase into water and oxygen, which manifests itself as a decrease in absorbance in the ultraviolet spectrum. This reduction in absorbance is directly proportional to the activity of the CAT enzyme (16).

GSH-Px activity was measured by the method of Paglia and Valentine (17). The enzymatic reaction was initiated by adding H_2O_2 to the reaction mixture containing reduced glutathione, reduced nicotinamide adenine dinucleotide phosphate, and glutathione reductase. The change in the absorbance at 340 nm was monitored by spectrophotometer. One unit of GSH-Px is defined as micromoles of NADPH oxidized per minute.

SOD activity measured by the method of Sun based on the changes adapted by Durak et al. (18); this method is based on the reduction of superoxide produced by the xanthine / xanthine oxidase system to nitro blue tetrazolium (NBT). Superoxide radicals (O_2^-), create a coloured farmazon by reducing the NBT in the medium, this complex gives maximum absorbance at 560 nm. A SOD unit is equal to the enzyme activity that inhibits NBT reduction by 50%.

TBARM, SOD, CAT, GSH-Px results were expressed in proportion to gr protein.

Biochemical measurements in serum

Serum total antioxidant capacity and total oxidant levels were determined by a commercially available colorimetric method (Rel Assay Kit Diagnostics, Turkey) developed by Erel, serum urea and creatinine levels and ALT and AST activity levels were determined by spectrophotometric method in an auto analyser (Abbot Architect C-8000, Germany).

Oxidative Stress Index (OSI); was calculated as TOS (µmol H_2O_2 equiv./lt) / TAS x 10 (mmol Trolox equiv./lt) and the results were expressed as arbitrary unit (AU) (19).

Histopathological Studies

Tissues taken for histopathological examination were kept in 10% formalin, paraffin-embedded section was taken, and sections were stained with Hematoxylin-Eosin (H&E) and Periodic Acid Schiff (PAS). The preparations were examined in NikonEclipseNi light microscope with Nikon DS-Ri2 digital camera using NIS-Elements 4.50 software program**.**

The interstisyel tubular damage was graduated according to the Goujon et al. (20). It based on 6 basic morphological patterns: 1) apical vacuolization; 2) tubular necrosis; 3) tubular dilatation; 4) brush border integrity; 5) cell detachment; 6) denuded basement membrane.

The morphological changes were graded on a 5-point scale: 1, no abnormality; 2, mild lesions affecting 10% or less of kidney samples; 3, lesions affecting 25% of kidney samples; 4, lesions affecting 50% of kidney samples; and 5, lesions affecting 75% or more of kidney samples.

Statistical analyses of the histology results were performed using SPSS (Version 22). The mean comparisons of more than 2 groups were tested by Kruskal-Wallis Variance Analysis. After the analysis of variance, post-hoc was used to determine the different groups and the Bonferroni test was used for paired comparisons. The statistical significance level was accepted as 0.05.

Statistical Analyses

Statistical analyses of the biochemical analysis were performed using MedCalc (Version 15.8) and the results were presented as Median and 25 P-75 P. The median comparisons of more than 2 groups were tested by Kruskal-Wallis Variance Analysis. After the analysis of variance, post-hoc was used to determine the different groups and the Bonferroni test was used for paired comparisons. The statistical significance level was accepted as 0.05.

RESULTS

TBARM levels decreased in all treatment groups compared to those of I/R group. Levels of TBARM in DHLA-applicated group were found close to control (Table 1).

CAT enzyme activities were higher in all treated groups compared to I/R group. Improvement in CAT activity was prominent in short-term DHLA group (Table 1).

GSH-Px enzyme activities significantly decreased in I/R group compared to control and improved in all treated groups compared to the I/R group. There was no significant difference between all treated groups (Table 1).

SOD levels were found to be significantly lower than control in all groups ($p<0.001$), there was no difference between the I/R group and the treated groups (Table 1).

TAS levels were significantly increased in I/R group compared to control. In all treated groups, the TAS levels were close to the control group and there was significant difference at treated groups with I/R ($p<0.001$) (Table 2).

TOS levels were significantly decreased in I/R group compared to control (Table 2).

OSI ındex significantly increased in I/R group compared to control ($p<0.001$), in all treated groups, the OSI levels were close to the control group and there is significant difference between treated groups with I/R group ($p<0.001$) (Table 2).

Histopathological results

In the histopathologic examination of our study, there was more damage in the internal part of the medulla and external cortical area tubule epithelium of the cortex in the ischemia-reperfusion group. An increase in intraglomerular

Table 1. Tissue TBARM levels and SOD, CAT, GSH-Px enzyme activities

TissueParameters		Control	I/R	Long-Term ALA+ I/R	Short-Term ALA+I/R	Short-Term DHLA+I/R
Thiobarbituric Acid Reactive Sunstances (TBARM) (nmol /mg protein)	(mean± SD),	0.39±0.05	1.36±0.17[a]	1.04±0.15[b]	0.72±0.09[c]	0.39±0.04
	(median±SD)	0.38±0.05	1.37±0.17	1.025±0.15	0.68±0.09	0.38±0.04
Superoxide Dismutase (SOD)(U/mg protein)	(mean± SD),	0.71±0.03	0.6±0.065[a]	0.60±0.06[a]	0.63±0.04[a]	0.60±0.07[a]
	(median±SD)	0.65±0.03	0.58±0.065	0.64±0.06	0.62±0.04	0.57±0.07
Catalase (CAT)(k /mg protein)	(mean± SD)	0.12±0.02	0.04±0.008[a]	0.07±0.01[b]	0.06±0.008[b]	0.09±0.01[c]
	(median±SD)	0.12±0.02	0.04±0.008	0.07±0.01	0.06±0.008	0.08±0.01
Glutathione Peroxidase (GSH-Px)(U /mg protein)	(mean± SD)	0.45±0.04	0.20±0.028[a]	0.376±0.05[b]	0.36±0.05[b]	0.37±0.04[b]
	(median±SD)	0.45±0.04	0.21±0.028	0.37±0.05	0.34±0.05	0.37±0.04

a, represent a significant difference between control and I/R. Different letters represent a significant difference between groups ($p < 0.001$).

Table 2. Serum TAS, TOS and OSI levels

Serum Parameters		Control	I/R	Long-Term ALA+I/R	Short-Term ALA+I/R	Short-Term DHLA+I/R
Total Antioxidant Status (TAS) (mmol/l)	(mean± SD),	1.5±0.07	1.27±0.05[a]	1.54±0.13[b]	1.70±0.08[b]	1.64±0.15[b]
	(median± SD)	1.52±0.07	1.27±0.05	1.49±0.13	1.71±0.08	1.64±0.15
Total Oxidative Stress (TOS) (umol/l)	(mean± SD),	19.8±2.1	38.1±6.37[a]	27.8±3.71[b]	31.5±7.16[a]	25.5±3.6[b]
	(median± SD)	20.6±2.1	36.76±6.37	28.73±3.71	31.78±7.16	25.41±3.6
Oxidative Stress İndex (OSİ) (arbitrary unit)	(mean± SD)	1.3±0.15	2.99±0.44[a]	1.80±0.21[b]	1.85±0.37[b]	1.56±0.21[b]
	(median± SD)	1.32±0.15	3.08±0.44	1.78±0.21	1.85±0.37	1.53±0.21

a, represents a significant difference between control and I/R. Different letters represent a significant difference between groups ($p < 0.001$).

and peritubular blood was observed. Edema was also present in the glomerular area. Vacuolization of the cell apical, tubular necrosis, tubular dilatation, brushy edge damage, denudation of tubular basal membrane, and intratubular filtration of dissociated cells were extensively present and significantly different compared to control. There was no statistically significant difference between I/R group and ALA treated groups. Only significant difference was observed between I/R and short-term DHLA groups (Table 3).

DISCUSSION

In this study, we aimed to compare the effects of short and long-term administration of ALA with short-term administration of DHLA on TBARM and antioxidant enzymes including GSH-Px, CAT and SOD activities in renal tissue. All tissues were also evaluated by histopathological evaluation. In addition, we evaluated total serum systemic oxidative stress (TOS) and antioxidant parameters (TAS). The present data demonstrate that both long and short-term administration

Table 3. Histopathological results

	(1) Mean±SD Median (min-max)	(2) Mean±SD Median (min-max)	(3) Mean±SD Median (min-max)	(4) Mean±SD Median (min-max)	(5) Mean±SD Median (min-max)	p values	Post hoc p values
(N)	0±0 0 (0-0)	2.71±0.48 3 (2-3)	2±0.57 2 (1-3)	2±0.57 2 (1-3)	1.29±0.48 1 (1-2)	<0.001*	**1-2: <0.001***
							1-3: 0.008*
							1-4: 0.008*
							1-5: 0.560
							2-3: 1.000
							2-4: 1.000
							2-5: 0.042*
							3-4: 1.000
							3-5: 1.000
							4-5: 1.000

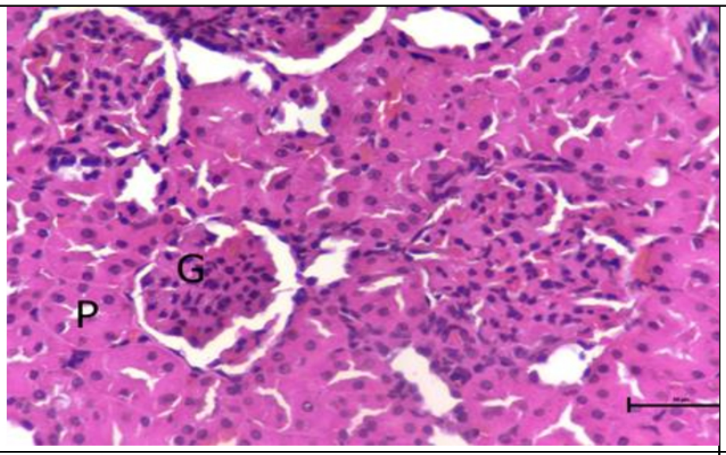
(1), control; (2) I/R; (3) Short-Term ALA+I/R, (4) Long-Term ALA+I/R; (5) Short-Term DHLA+I/R, N = 1) apical vacuolization; 2) tubular necrosis; 3) tubular dilatation; 4) brush border integrity; 5) cell detachment; 6) denuded basement membrane. * Kruskal-Wallis test

of ALA and short-term DHLA significantly reduced oxidative damage in renal tissue and improved systemic oxidative stress parameters. However, short-term DHLA effects on tissue CAT activity and TBARM levels were more prominent at the tissue level.

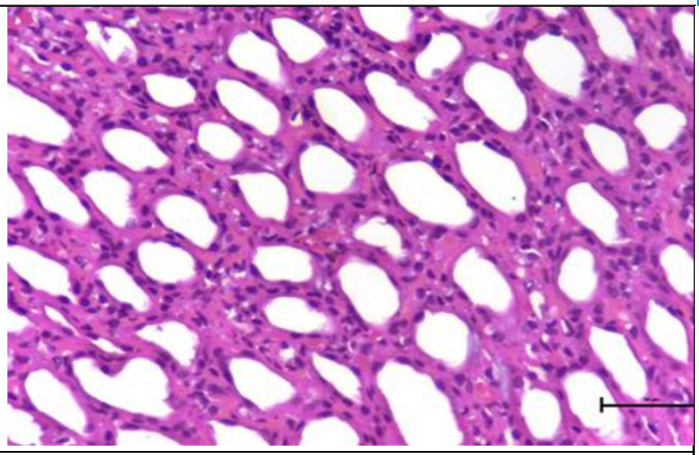
There are many studies showing the protective effects of ALA on I/R damage in various tissues including renal tissue. However, there is no study in literature investigating DHLA effect, which is reduced form of ALA, on renal I/R model.

Renal ischemia is an important clinical pathology seen in many clinical conditions such as renal transplantation, partial nephrectomy, cardiopulmonary bypass, sepsis, various urological interventions and hydro nephrosis. Reperfusion is another contributing factor to tissue damage in these conditions. Although reperfusion is essential for the survival of ischemic tissues, reperfusion itself, after a period of ischemia, increases the tissue damage. Reperfusion injury is attributed to the generation of ROS (21). Increasing ROS level ultimately leads to lipid peroxidation in cell membranes and forms the final product of lipid peroxidation, malondialdehyde (MDA) and lipid hydro peroxides (Thiobarbituric acid reactive sunstances; TBARM) (22).

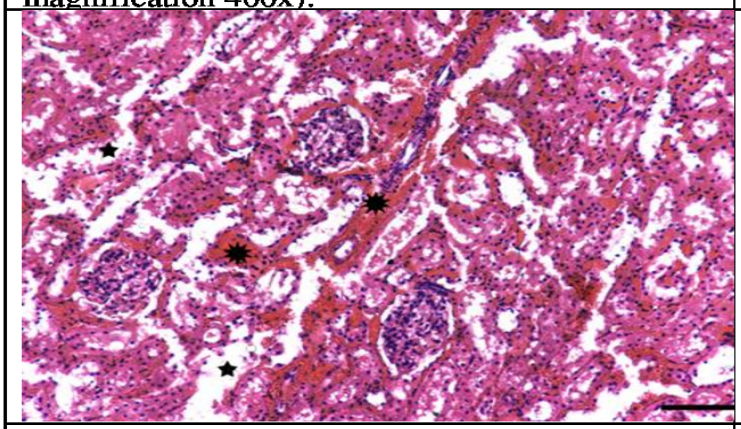
In this study, we found that TBARM levels increased significantly in the I/R group and improved significantly after both long and short-term ALA and short-term DHLA administration (Table 1, $p<0.001$). In many studies, it has been shown that ALA has an improving effect on tissue TBARM levels caused by I/R injury. Cosar et al. showed that ALA decreased both tissue and plasma MDA levels in ovarian I/R injury (23). In another study, Sehirli et al. found that ALA reversed I/R induced oxidant response and improves microscopic damage and renal function. They also speculated that ALA protects renal tissues by inhibiting neutrophil infiltration, balancing the oxidant–antioxidant status, and regulating the generation of inflammatory mediators (24). Wongmekiat O. et al. observed the reduction of oxidative product MDA and the recovery effect on antioxidant capacity in the obstructed kidney treated with ALA (25). ALA is also recognized as a universal antioxidant capable of scavenging free radicals, chelating metals, regenerating endogenous antioxidants, and modulating various signal transduction pathways (26). In the current study, our results were compatible with previous studies. The decrease in tissue TBARM levels can be related to the scavenging effects of ALA. However, the improving effect was prominent in the short-term DHLA treated group (Table 1). It is known that DHLA is the reduced form of ALA. In cells containing mitochondria, α-lipoic acid is reduced by NADH-dependent reaction to dihydrolipoic acid. To carry out its function, the disulphide group of lipoic acid is reduced to its dithiol form, DHLA, which is an active form of ALA (27). In challenging conditions such as ischemia or I/R injury, the conversion of ALA to DHLA may be adversely affected because of the higher cytosolic redox state observed in these conditions. DHLA is active against free radicals such as hydroxyl radicals and singlet oxygen. It also reduces the oxidized form of vitamins including ascorbic acid and vit E. ALA and DHLA work similar to that of the glutathione-reduced glutathione pairs (13). Superoxide radical and hydrogen peroxide which are not scavenged by α-lipoic acid itself but by its reduced form, DHLA (28). Therefore, we suggest that



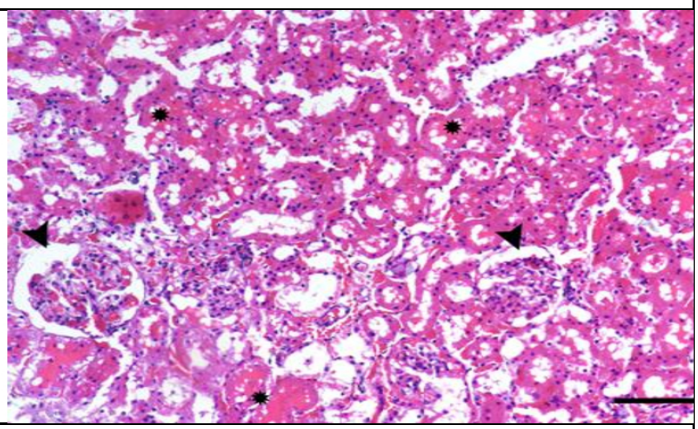
1a: Glomeruli (G) and proximal (P) tubules in renal cortex of controls (H&E, original magnification 400x).



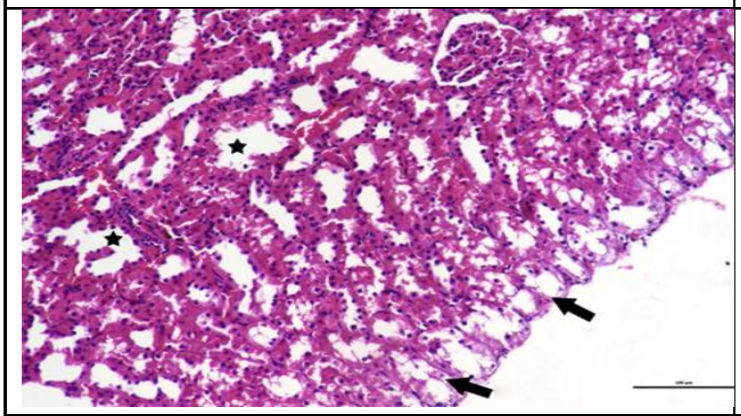
1b: Medullar tubules in controls (H&E, original magnification 400x).



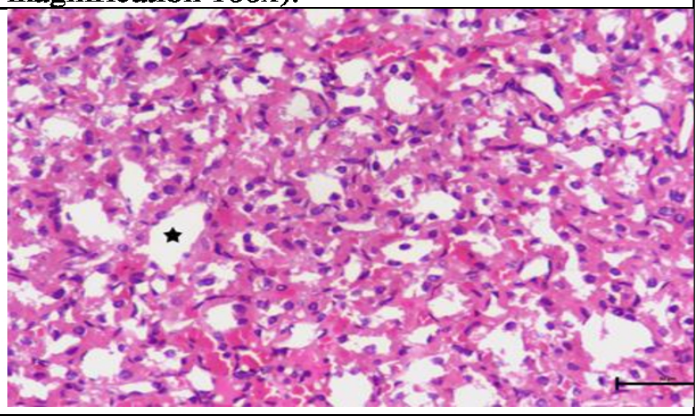
1c: Common hemarogical areas (asterix) and dilated tubules (star) in I/R group. (H&E, original magnification 400x).



1d: Hemarogical area (asterix) and glomerulus with dilated Bowman's space (arrow head) in long-term ALA+I/R group (H&E, original magnification 100x).



1e: Denudated tubular epithelium (arrows) and dilated tubules (asterix) in short-term ALA + I/R group (H&E, original magnification 100x).



1f: Degeneration and dilatation (star) at tubular epithelial cells in the medullar region in short-term DHLA group (H&E, original magnification 400x).

direct administration of DHLA may be more and rapidly effective in I/R damage.

Antioxidant enzymes, mainly SOD, CAT, and GSH-Px, are well-known defense mechanisms to prevent oxidative damage by specific free radical groups in tissue levels. Sivaprasad et al. investigated the protective effects of ALA (25 mg / kg-day, in), on CAT, GSH-Px, and SOD activities in mouse liver damage by lead intoxication. They found that ALA caused a significant increase in all three enzymes in tissue level (29). They attributed these effects to the direct scavenging role of ALA on oxygen-free radicals and increased GSH production.

In the current study, we also evaluated antioxidant enzyme activities in tissue levels and found that CAT, GSH-Px, and SOD levels in the I/R group were significantly lower than the control group ($p<0.001$). CAT and GSH-Px levels were increased significantly in the long and short-term ALA ($p<0.001$). However, we observed a similar improving effect of short-term DHLA on GSH-Px activity ($p<0.001$) compared to other treated groups. Accordingly, our results suggest that the short-term administration of DHLA may have a curative effect on antioxidant enzymes. It is known that a continuous source of reduced glutathione is needed for GSH-Px enzyme to function and DHLA has been shown to be a potent reductant as ALA and directly and indirectly regenerate glutathione (30,31).

However, the improving effect on CAT activity was prominent in short-term DHLA treated group. This improvement paralleled the decrease in the TBARM levels in the same group.

While there was no significant change observed in SOD levels in all treated groups. The antioxidant enzymes can be affected at different levels due to different factors related to gene regulation or epigenetic mechanisms. In a study, it was suggested that the expression of antioxidant genes during ischemia-reperfusion are not coordinately expressed and that the differential loss of antioxidant enzymes may be the contributing factor(s) towards the heterogeneous renal tissue damage due to I/R induced oxidative stress (32). In the current study, one of the reasons why enzyme activities are affected differently may be related to the mechanism described above.

In our study, serum TAS and TOS levels were also assayed to observe the systemic effects of ALA and DHLA. Serum OSI values were also calculated, which reflects the relative change in the TAS and TOS values and a better indicator of systemic oxidative balance. OSI values in the I/R group were found to be significantly higher than the control group ($P <0.001$). OSI values were improved in long and short-term ALA and short-term DHLA treated groups and there was no significant change observed between treatment groups. Therefore, it can be said that long and short-term use of ALA and short-term use of DHLA have a healing effect on oxidative balance not only tissue level but also systemic level.

In our study, the histopathological effects of ALA and DHLA on renal tissue were also evaluated. Takaoka M. et al. (2002) investigated the protective effect of alpha-lipoic acid against ischemic acute renal failure in rats. They were observed tubular necrosis and medullary obstruction in the acute renal failure group, and tubular necrosis and medullary obstruction were suppressed in the lipoic acid treatment group (21).

In the current study, cortical tubular structures were deteriorated in I/R group compared to the control (Figure 1a-c, $p<0.001$), in accordance with previous studies (33, 34). These pathological changes were not significantly improved in long-term and short-term ALA treated groups in terms of histological scoring (Figure 1d) but partially recovered in short-term DHLA group (Figure 1f). It can be said that dramatic effects were observed in antioxidant enzyme activities and systemic total oxidative stress parameters but not at the tissue level. A longer time may be required before the healing effects on tissue level can be observed. Histopathological effects were compatible with the tissue TBARM, CAT, and GSH-Px levels only in short-term DHLA group. It can be attributed to the DHLA being more effective than ALA, which must be converted to DHLA for its effects.

CONCLUSION

In conclusion, long and short-term ALA and short-term DHLA administration have significant improving effects on TBARM levels in renal I/R injury and the loss of antioxidant CAT and GSH-Px enzyme activities in tissue levels were significantly recovered. Both substances have a potential for treatment. However, DHLA administration is more effective on TBARM and CAT enzyme activities compared to ALA. DHLA effects were also prominent at the tissue level and can be preferred to ALA in clinical pathologies associated with I/R condition.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

Ethical permission was obtained from the Hatay Mustafa Kemal University, Animal Research Ethics Committee for this study with date 27/10/2015 and number 2015/8-2, and Helsinki Declaration rules were followed to conduct this study.

Authors Contributions

Concept: F.K., O.Ö., Design: F.K., O.Ö., H.S.B., S.G., Data Collection or Processing: F. K., O.Ö., E.A., H.S.B., Analysis or Interpretation: F.K., O.Ö., A.A., E.A., H.S.B., Literature Search: F.K., O.Ö., Writing: F.Ka., O.Ö., A.A., E.A., S.G.

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