

EFFECT OF ALPHA LIPOIC ACID ON RAT KIDNEYS
IN ACITRETIN AND METHOTREXATE INDUCED
OXIDATIVE INJURY

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Abstract

This experimental study aimed to biochemically evaluate the possible protective effect of alpha lipoic acid (ALA) on the kidneys in acitretin (ACT) and methotrexat (MTX) induced damage and toxicity mechanisms. A total of 51 rats weighing 260–420 g were used for the study. The animals were divided into the following four groups: control, ALA, ACT and MTX, and ACT+MTX+ALA. On days 3, 5 and 7 after the injection of the rats, a total of 45 rats were sacrificed by cervical dislocation under general anesthesia. The kidney tissue samples for biochemical analysis were taken. Malondialdehyde (MDA) level in ALA group showed a non-significant decrease as compared to the control group and it was significantly higher as compared to the ACT+MTX group. A significant increase was also observed compared to the ACT+MTX+ALA group. Catalase (CAT) and Manganese superoxide dismutase (MnSOD) levels increased in all groups as compared to the control, but this increase was not significant. Furthermore, superoxide dismutase (SOD) and glutathione reductase (GSR) decreased in all groups as compared to the control group, although this difference did not reach statistical significance. Further research using different doses of ALA would be needed to clarify the details of the effect of ALA on the MTX-ACT combination.

Key words: alpha lipoic acid, kidney, methotrexate, protective effect, acitretin

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Introduction. Acitretin (ACT) belongs to a group of drugs called synthetic retinoids, which are similar to vitamin A and are active metabolites of etretinate [1]. In addition to regulating cell proliferation and differentiation, it also has apoptosis and inhibition of tumour development and anti-inflammatory effects. It differs from other systemic antipsoriatic agents in that it is not immunosuppressive and cytotoxic. However, it is not recommended for use in women of childbearing age as long as an alternative is available [2]. However, while the use of acitretin is an effective method in the treatment of psoriasis, side effects can also occur, such as cheilitis, hyperlipidemia, dry skin-nose-eye-mouth, nail disorders, thinning of hair, elevated transaminases, and hepatotoxicity [1].

Methotrexate (MTX) is chemical 4-amino N10-methyl analogue of folic acid acting as a folic acid antagonist. MTX has strong immunomodulatory activity against psoriasis, inflammatory bowel disease, and inflammatory arthritis. MTX is also used as a chemotherapeutic agent in the treatment of some types of cancer, such as lung, breast, acute lymphoblastic leukemia, and head and neck cancer [3]. However, MTX can cause toxicity in some cases. Along with affecting target cells, MTX also affects healthy cells and can be more toxic than other drugs. In previous research, it was observed that MTX causes toxicity on the liver, kidneys, lungs and gonads [4].

As part of a combined treatment, ACT has been frequently used in combination with MTX in the treatment of psoriasis. Yet, this combination can cause negative effects [4]. To date, few studies have investigated the possible damage to kidney tissue caused by the combination of MTX with ACT, or by the addition of α -lipoic acid (ALA) as an antioxidant to this combination and the protective effect of ALA on this damage.

ALA is a naturally occurring antioxidant found in many prokaryotic and eukaryotic cells that acts as a cofactor in mitochondrial enzymes. ALA has important functions such as scavenging free radicals and protecting the cells against oxidative stress by removing intracellular ROS. In addition, ALA has properties such as preventing lipid peroxidation, chelating with free metal ions, neutralizing free radicals [5].

This study aimed to biochemically evaluate the possible protective effect of ALA on the kidneys in ACT and MTX-induced damage and toxicity mechanisms. Another goal was to explore the role of ALA as an important antioxidant by regenerating cellular antioxidants.

The study was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (2018/13).

Materials and methods. Animals. A total of 51 Wistar albino type male rats (weighing between 260 and 420 g) were used. The animals were obtained from the Ondokuz Mayıs University Experimental Animal Application and Research Centre (DEHAM) and fed with standard mouse food and water.

Experimental groups and test protocols.

1. Control (C) group: No procedure was applied to the rats in this group (six rats were used in the control group).
2. ALA group: ALA (50 mg/kg/day) was given to each rat by intraperitoneal (i.p.) injection. ALA was administered daily at the same time in the morning.
3. ACT+MTX group: ACT (20 mg/kg/day) and MTX (20 mg/kg/day) were administered to each rat by i.p. injection. MTX was administered on the second day of the injection.
4. ACT+MTX+ALA group: ACT, MTX and ALA were given to each rat by i.p. injection. MTX was administered on the second day of the injection.

There were 15 rats in each of Groups 2, 3, and 4. ACT, MTX and ALA were dissolved in 0.9% NaCl. Their combinations were administered i.p. to the rats on the body weight level [6].

Tissue collection, homogenization and preparation for biochemical analysis. On days 3, 5, and 7 post-injection, a total of 45 rats from Groups 2–4 (five animals per group) were sacrificed by cervical dislocation under general anesthesia, control group sacrificed on day 7. Next, the kidneys were removed bilaterally. Cold phosphate buffer solution was added at a ratio of 1/10 (w/v) to the tissue samples taken into plastic tubes. The tissue samples were finely chopped in phosphate buffer by surgical scissors. The tissue samples were then homogenized 3 times at 8000 rpm for 20 s. They were homogenized six times at 25% amplitude for 20 s with 10-second intervals in an ultrasonication device. All these operations were carried out in iced environments. The obtained homogenates were centrifuged at 15 000 rpm for 15 min at +4 °C. The supernatants were separated for enzyme activity measurements. After preparation, the tissue homogenates were stored at –80 °C until biochemical analyses were performed to preserve enzyme activity and prevent oxidative degradation.

Biochemical analysis. MDA level. Malondialdehyde (MDA) method is based on the reaction of MDA with thiobarbituric acid, which forms a pink pigment with maximum absorbance at 532 nm at acid pH. The results were expressed as $\mu\text{mol}/\text{mg}$ protein concentration [7].

SOD and MN-SOD activity. Pure SOD enzyme incubated with KCN was used to determine Mn-SOD activity. The optical density of the formed red product was read at 550 nm. The results were expressed as IU/mg protein [8].

Catalase activity. The principle of this method is based on the determination of the rate of dissociation of H_2O_2 to H_2O under the influence of CAT at 240 nm [9].

Glutathione reductase activity. GSR activity was measured by spectrophotometrically monitoring the oxidation of NADPH in the presence of NADPH at 340 nm at 37 °C [10].

Statistical analyses. Statistical analysis was performed using the SPSS version 22.0 (IBM Corp, Armonk, NY, USA). The descriptive statistics were expressed in mean±standard deviation (SD). The data were compared between groups using Kruskal–Wallis analysis of variance or two-way ANOVA according to their normality. A *p* value less than 0.05 was considered statistically significant.

Results. MDA levels, SOD, CAT, MnSOD and GSR activities in supernatants obtained from rat kidneys are summarized in Table 1. For the MDA level in ALA group, a non-significant decrease (*p* = 0.421) as compared to the control group was observed and it was significantly higher as compared to the ACT+MTX group (*p* = 0.00). Furthermore, a significant increase was also observed as compared to the ACT+MTX+ALA group (*p* = 0.00). CAT and MnSOD levels were found to increase in all groups as compared to the control; however, this increase did not reach statistical significance (*p* = 0.586; *p* = 0.241, respectively). SOD and GSR decreased in all groups as compared to the control group and this difference was not significant (*p* = 0.088 and *p* = 0.076, respectively).

T a b l e 1

Antioxidant enzymes activities of SOD, Mn-SOD, MDA, CAT and GSR in rat kidney tissue

Groups	MDA mean±SD	CAT mean±SD	SOD mean±SD
1. Control group	4.57±0.10	5.83±4.59	4.64±4.0
2. ALA group	3.39±0.46 ^{a***}	7.78±3.43 ^{a***}	3.35±1.83 ^{a***}
3. ACT+MTX group	5.01±0.48 ^{b**}	6.60±4.03 ^{a***}	1.84±1.31 ^{a***}
4. ACT+MTX+ALA group	6.79±1.89 ^{b**}	7.88±2.76 ^{a***}	3.69±2.72 ^{a***}
Groups	MNSOD mean±SD	GSR mean±SD	
1. Control group	0.99±1.08	6.6±2.40	
2. ALA group	1.91±1.51 ^{a***}	4.10±2.11 ^{a***}	
3. ACT+MTX group	2.94±2.20 ^{a***}	4.97±4.27 ^{a***}	
4. ACT+MTX+ALA group	1.65±0.92 ^{a***}	5.79±2.88 ^{a***}	

Note: According to both Two-way ANOVA and Kruskal–Wallis analyses, the effect of time (day 3, 5, and 7) was not statistically significant for any parameter (MDA, CAT, SOD, MnSOD, and GSR) (*p* > 0.05) (*p* = 0.608; *p* = 0.619; *p* = 0.309; *p* = 0.599; *p* = 0.198, respectively). Therefore, values are presented as group means±SD.

^a: Comparison with the control group; ^b: Comparison with the ALA group

p* < 0.05, *p* = 0.00, ****p* > 0.05

Discussion. Although chemotherapeutic agents are used for treatment purposes, they can cause undesirable effects in different tissues. There are not many studies related to the effects of ALA on renal damage that can be caused by the

combination of MTX and ACT. In this study, it was suggested that ALA may have ameliorative properties against potential side effects that may occur as a result of the combination of MTX and ACT. In the study, the possible protective effects of ALA, which has anti-inflammatory and antioxidant properties against possible damage in renal tissues in rats induced by ACT and MTX, were investigated.

The organism has a complex antioxidant defense system, both enzymatic and nonenzymatic. Antioxidant defense systems act against free radicals which have a harmful effect on tissues in the body. In this way, they clean free radicals and prevent cell damage. SOD, CAT, glutathione peroxidase (GPx) and GR form this defense line in the cell concentration [11].

ACT is a retinoid and analogue of vitamin A that regulates cell proliferation and differentiation; in addition, it is also a teratogenic drug. ACT is used to treat psoriasis. However, while the use of acitretin is an effective treatment method, side effects can also occur, such as cheilitis, hyperlipidemia, elevated transaminases, and hepatotoxicity. Its adverse effect on the kidneys has not been extensively reported yet [1].

MTX, which is used in the treatment of many diseases, has toxic effects on some tissues. While MTX increases ROS and nitrogen production, it was also reported to reduce glutathione, SOD and CAT levels. Based on this evidence, it was suggested that MTX causes mitochondrial damage, lipid peroxidation, and reduces the effectiveness of antioxidant defense systems. The toxic effect of MTX on cells has not only been reported in cancer cases [11].

KABAKÇI et al. [12] observed that MDA levels significantly increased with MTX compared to the sham group. The authors also documented that CAT and SOD enzyme levels decreased in the kidney tissue as compared to the control. In addition, the authors also detected serious morphological damage. CHAUHAN et al. [13] also observed that MTX increased MDA levels and decreased GSH levels.

As stated in previous studies, in most experimental studies conducted with MTX, MDA levels increased after MTX application, while enzyme activities such as CAT, GSH, GPx, CAT and SOD were reported to decrease. Similarly, in our study, the amount of MDA, CAT, and MnSOD in the kidney tissue of rats increased with MTX application and in addition to ACT administered in combination. In addition, a decrease in SOD and GR levels was observed.

ALA is a natural compound acting as a cofactor in mitochondrial enzymes. This compound also exhibits antioxidant properties. When taken orally, over 93% is absorbed by the small intestine and then reaches the liver. Furthermore, 80% of the ALA can be excreted through urine [11].

HASAN and ALGAREEB [14] observed that MTX decreased SOD and GSH levels in the liver tissue of mice and increased MDA levels. Later, when these rats were given ALA, SOD and GSH levels increased, while MDA levels decreased. SALAMA et al. [15] investigated the effect of ALA on liver damage induced by thioacetamide. The results revealed that GST and GSH levels decreased, while

MDA levels increased in the thioacetamide group. After ALA was given to the treatment group, GST and GSH levels increased, while MDA levels decreased.

In a study investigating the effect of ALA against the effects of lead on the liver and kidney of toadfish *Halobatrachus didactylus*, CAMPANA et al. [16] found that ALA has a positive effect on the liver, but no effect on the kidneys.

YUCEL et al. [17] observed that ALA treatment after sciatic nerve compression injury had no therapeutic effect on oxidative stress and functional recovery. In a study on the mechanisms of antioxidant effects of ALA in diabetic and non-diabetic kidneys BHATTI et al. [18] observed that ALA improves albuminuria and pathology in diabetes by reducing oxidative stress; however, in healthy animals, it contributed to renal dysfunction.

ELSAWY et al. [11] used naringin antioxidant against MTX-induced nephrotoxicity. The authors observed an increase in GSH, SOD, CAT, and GPx activities. Another study examined the effects of ALA in the testicles against the toxic effect of MTX. The results revealed that, with the application of MTX, MDA levels increased while SOD, CAT and GPx levels decreased. As a result of ALA application, SOD, CAT, and GPx increased while MDA levels decreased [19]. Similarly, ABDEL-DAIM et al. [20] conducted a study to reduce hepatorenal toxicity that may occur due to MTX using diosmin. In the results, the authors observed increase in GSH levels; it was thus reported that there was an increase in SOD, CAT, and GPx enzyme activities.

In the present study, we observed an increase in CAT, SOD and GSR levels with the addition of ALA to the combination of ACT and MTX. We also found that ALA showed an antioxidant effect against the toxic effect caused by MTX and ACT. This result is similar to those of numerous previous studies conducted with ALA. However, despite the use of ALA, we observed an increase in MDA levels. One possible reason for this may be that the defense was insufficient in the formation of intense oxidative stress caused by MTX [21]. This result is similar to those previously reported by the studies conducted by Yucel [17] and Campana [16], in that ALA did not show a protective effect. In this sense, our results coincide with those previously reported by Bhatti et al. [18] in terms of its negative effect.

Conclusions. In this study, the increased CAT and MnSOD activities served as an indication that the amount of reactive oxygen species in both the cytosol and mitochondria of kidney cells increases when MTX and ACT are used together. However, when ALA was added to the MTX and ACT drugs combination, we found that CAT and SOD enzyme activities were triggered in the cytoplasm of kidney cells due to the effect of ALA. The inhibition of MnSOD activity in mitochondria was associated with the radical scavenging behaviour of ALA. In other words, when ALA was used together with this combination, it triggered an activity of radical scavenging enzymes in the cytoplasm and directly scavenged reactive oxygen species in the mitochondria. The decreased amount of MDA as

compared to the control group when ALA was given alone demonstrated its antioxidant property. In our study it did not show a statistically significant effect of the time factor across days.

This study is among the limited number of investigations that simultaneously evaluate the oxidative stress response in kidney tissue induced by the acitretin-methotrexate (ACT-MTX) combination and the biochemical effects of adding α -lipoic acid (ALA) to this setting, despite the frequently reported hepatotoxicity in the literature. In particular, the experimental demonstration of ALA's potential protective role against ACT-MTX-associated renal oxidative damage represents a significant novelty that distinguishes this study from previous reports. In this regard, the study provides a new biochemical perspective on the safety profile of ACT-MTX treatments.

Authors' contribution. E.D. designed the study. E.D., B.O. and F.G.S. performed the experimental studies and analyzed the data.

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