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The investigation of the effect of alpha lipoic acid on lung polyphenol oxidase activity in acitretin and methotrexate given rats

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Abstract: Oxidative stress is one of the main causes of lung damage caused by methotrexate (MTX). In addition, it has been observed that it causes serious side effects on other organs. In recent years, MTX has started to be used in therapy in combination with Acitretin (ACT). Alpha lipoic acid (ALA), which has antioxidant and anti-inflammatory activities, is naturally found in human foods. In this study, it was aimed to investigate the effect of ALA on phenol oxidase activity in lung tissue in the elimination of cellular level damage by free radicals produced by ACT and MTX. In the study, 50 Wistar albino male adult rats, selected from the same generation and weighing between 200 and 250 g, were used. The rats used were fed with standard mouse food and water was given free. Study groups were formed as control group (K), ALA group, ACT + MTX group and ACT + MTX + ALA group. ACT (20mg / kg / day), MTX (20mg / kg / week), ALA (50mg / kg / day) were dissolved in 0.9% NaCl and given to rats intraperitoneally. Polyphenol oxidase (PO) (1.10.3.1.) activation, which catalyzes the oxidation of phenolic compounds, was determined by the Hung and Boucias (1996) method. Compared to the control group, PO activity was found to be 66% higher in the MTX + ACT group and 46% higher in the MTX + ACT + ALA group. On day 5, the PO activity of the MTX + ACT + ALA group was 55% lower than in the MTX + ACT group, and this decrease was found statistically significant ($p < 0.05$). It is determined that this decrease has reduced to 20% on the 7th day. In the study, it was observed that the combined use of ACT and MTX increased phenol oxidase activity in rat lung tissue. A decrease in this activity was observed with the addition of an antioxidant ALA to this combined application.

Keywords: Alpha Lipoic Acid, Acitretin, Methotrexate, Phenol Oxidase

1 Introduction

Polyphenol oxidase (1,2-benzenediol:oxygen oxidareductase; EC 1.10.3.1) is an enzyme, which catalyzes the oxidation of phenolic compounds with molecular oxygen. These enzymes contain copper at their active site. This metal ion enables them to oxidize the phenolic group of an aromatic compound to a reaction group known as a Quinone (Whitaker 1995). This enzyme is found in many plant tissues, in some fungi, and in some higher animals, including insects and humans. (Wu et al. 2008). Polyphenol oxidase frequently called tyrosinase, phenolase, polyphenolase, catecol oxidase, cresolase or catecholase. The name *tyrosinase* came from tyrosine being used first as a substrate. It seems preferable to reserve the name tyrosinase for the mammalian enzyme, which has more limited specificity in that it acts only on tyrosine and dihydroxyphenilalanine. A number of important diseases in humans result from lack of sufficient activity or too much activity (Whitaker 1995). 4-hydroxyanisole a substrate of tyrosinase, was found to cause regression of Harding-Pasey melonama in mice. Another important area in which tyrosinase is used is the production of L-DOPA used in the treatment of Parkinson's disease (Espin et al.1997).

ACT is a second generation of synthet).ic mono-aromatic retinoid, pharmacologically the active metabolite of etretinate (Ormerod et al. 2010). ACT has the potential to inhibit and control some processes such as cell differentiation and proliferation, inflammation and keratinization (Pilkington and Brogden 1992). Retinoids affect the development and differentiation of epidermal cells, as well as the activity of adipose tissue by acting on the nuclear receptors of these processes Like all retinoids, ACT has also teratogenic effect (Ling 1999). Previous researches showed that after treatment with ACT, most patients have many side effects and pathology of various organs occurs (Katz et al. 1999 ; Bhuiyan and Chowdhury 2016).

MTX is a drug whose chemical structure resembles that of folic acid and which is able to block cell metabolism (Seideman et al. 1993). Methotrexate has been used in the treatment of many autoimmune diseases because it has folate antagonistic, anti-inflammatory and anti-proliferative

properties (Gerards et al. 2003). It is also used in lung, breast, acute lymphoblastic leukemia, lymphoma, head and neck cancers because MTX inhibits cell division (Gerards et al. 2003). MTX can indirectly prevent the synthesis of purine bases, which are necessary in the synthesis of DNA, RNA and adenosine phosphate by inhibiting the activity of tetrahydrofolate enzyme. Thus, the regeneration of cells becomes difficult which resulting in cell death (Jeffes and Kaneshiro 1998). Therefore, MTX has been used to treat various types of malignancy (cancer). Today, they are increasingly used for treatment in dermatology and rheumatology especially in psoriasis, psoriatic arthritis and rheumatoid arthritis (Lagarce et al. 2015).

MTX and ACT are known to have a healing effect in the treatment of psoriatic lesions. However, the combination of ACT and MTX is very rare in the treatment of psoriasis because the interaction of drugs leads in particular to hepatotoxicity (Carretero et al. 2013). The treatment of psoriasis should be taken in drug combinations against these side effects. It is recommended to take antioxidants when combine use is necessary (Karadağ et al. 2015).

ALA, which is found naturally in human foods, has antioxidant and anti-inflammatory activities (Rochette et al. 2013). ALA may have functions such as regeneration of vitamins C and E, protection against lipid peroxidation and free radicals, increasing SOD and CAT enzyme activities, repairing molecular damage, increasing acetylcholine production (Suh et al. 2005) In recent years, although the side effects are known, the use of the combination ACT + MTX in treatment has increased (Carretero et al. 2013). In the light of this information, in this study, the effect of ACT+MTX combination on polyphenol oxidase enzyme activity in rat lung tissue and whether it has protective role of antioxidant ALA was investigated.

2 Materials and Method

In this experimental study, 50 Wistar albino type male rats (weighing between 250 and 300 g) were used. All the animals, which were maintained for 12 hours in light and 12 hours in the dark and at a temperature of 21° C, were obtained from Ondokuz Mayıs University, Experimental Animals Application and Research Center (DEHAM). The studies were approved by the Ethics Committee of Ondokuz Mayıs University (2018/ 13). The rats were fed with standard mouse food and water was given unlimitedly.

The organizations of experimental groups were as follows:

Group 1 (C group) : The control group is the group that has not been treated.

Group 2 (ALA group): received 50 mg/kg/day of ALA;

Group 3 (ACT + MTXgroup): received 20mg/kg/day of ACT and also received 20mg/kg/week of MTX;
Group 4 (ACT + MTX + ALA): received 20mg/kg/day of ACT, 20mg/kg/week of MTX and 50mg/kg/day of ALA.

There are 15 rats in each of Group 2, Group 3, Group 4 groups that make up the experimental groups. Rats were starved for the last 24 hours before injection. Injection procedures were carried out at the same time every morning. ACT, MTX and ALA were resolved in 0.9 % NaCl, ACT (20 mg /kg /day) (An et al. 2017), MTX (20 mg /kg /week) (Jingang et al. 2017), ALA (50 mg / kg / day) (Maritim et al. 2003) and their combinations were given to the rats as intraperitonal injection (i.p) at the body weight level of each. From each the of experimental groups, 5 rats on the 3rd day, 5 on the 5th day and 5 on the 7th day following injection were sacrificed by cervical dislocation by giving general anesthesia.

Rats were given 50 mg/kg ketamine HCl (ketalar) and 10 mg/kg Xylazine (Rompul) as general anesthesia. Following this, perfusion was performed with 0.9 % NaCl and lungs were removed. The lungs were placed in containers containing isotonic sucrose and stored in a freezer at -80 ° C for experimental procedures. After the lungs removed from the freezer were thawed, their weights were determined by weighing them on a sensitive scale. Then, homogenization, sonication and centrifugation processes were carried out according to the procedures. The supernatants obtained after centrifugation were used for analysis.

The activity of polyphenol oxidase in lung homogenates was determined by the Hung and Boucias (1996) method. 50 µL homogenate was rapidly added to 950 µL phosphate buffer solution containing 20 mM L-DOPA. Then, the activity was determined by reading the absorbance change in a minute against the curve at 420 nm. 1 Enzyme Unit: It was defined as a 0.001 increase in 1 minute in the tub where the reaction occurred.

All data were evaluated using SPSS 22.0 statistical software. The difference between groups was checked with Kruskal Wallis test.

3 Results

Compared to the control group, it was determined that phenol oxidase activity increased in the group given ALA. This increase in activity; While it was 24% ($p > 0,05$) on the 3rd day, it doubled on the 5th day. On the 7th day, it was observed that the 5th day activity increased approximately 10% ($p > 0,05$) (Fig 1).

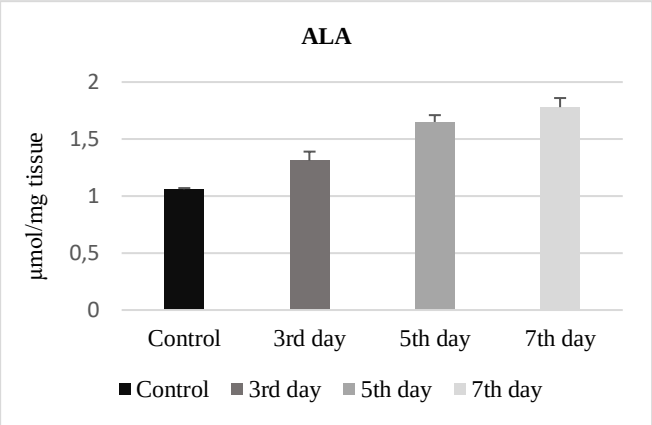

Fig. 1 Changes in Phenol Oxidase activity in the group receiving ALA

When MTX + ACT group is compared with the control group; While phenol oxidase activity increased 66% ($p <$

0,05) on the 3rd day, it was observed that it reached almost 100% ($p < 0,05$) on the 5th and 7th days (Fig 2).

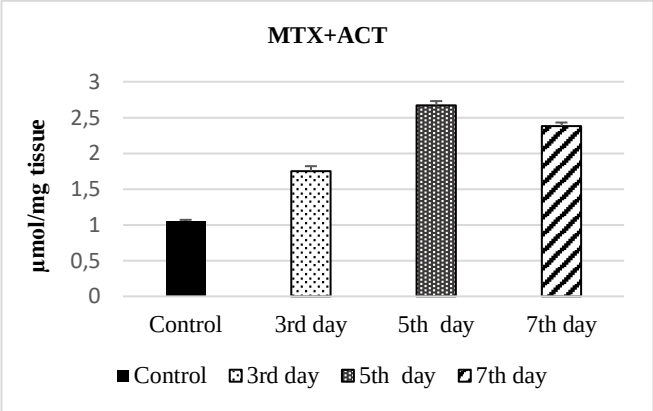

Fig. 2 Changes in Phenol Oxidase activity in the group receiving MTX+ACT

When MTX + ACT group is compared with the control group; Phenol oxidase activity increased 36 % ($p < 0,05$) on the 3rd day, % 12 ($p>0,05$) on the 5th % 82 ($p < 0,05$) on the 7th days (Fig 3).

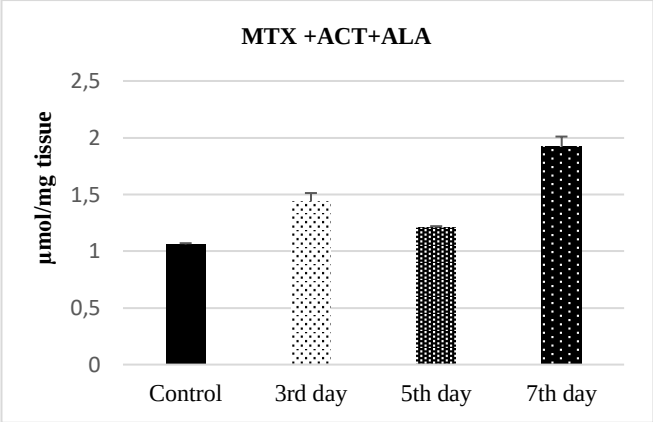

Fig. 3 Changes in Phenol Oxidase activity in the group receiving MTX+ACT+ALA

Phenol oxidase, which shows max activity on the 5th day in the MTX + ACT group; It was observed that reduced by 55% ($p < 0,05$) with the addition of ALA group (Fig 4).

4 Discussion

This study investigated the effect of acitretin and methotrexate application on phenol oxidase enzyme activity in rat lung tissues and as a result of adding alpha lipoic acid to this combination. Preview study has shown that oxidative stress caused by MTX plays a major role in tissue damage and these damages are reduced by ALA (Arpag et al. 2018).

MTX and ACT are widely used in the treatment of psoriasis. However, some case reports have shown that many side effects, especially acute interstitial pneumonitis, pulmonary fibrosis, occur after treatment with MTX (Bartram, 1998; Chikura et al. 2008).

In a study conducted by Armagan et al. in 2015, an increase in MDA level occurred in the group where MTX was given in kidney tissues compared to control. In the treatment group that received MTX + ALA, this increase decreased. It was concluded that ALA is effective with antioxidant and other properties in preventing the toxic effect of MTX in kidney tissue.

In the study of Moungjaroen et al. (2006), it has shown that ALA can produce reactive oxygen species that support ALA-related cell death in lung cancer. Gerritsen et al. (1994) explain that tyrosinase shows genoprotective activity by hydroxylation of tyrosine to L-DOPA and oxidation of L-DOPA to quinone. Han et al. (1996) stated that L-DOPA regulates the cellular antioxidant defense mechanism under certain conditions. Tyrosinase, peroxidase and laccase, members of the polyphenole oxidase enzyme family, have been investigated to remove toxic chemicals from drinking water and industrial wastewater (Wada et al 1992, Wada et al. 1993). However, many studies have been conducted, but activity studies in human metabolism have been limited. In our study, it was observed that MTX + ACT combination increased phenol oxidase activity in rat lung and with the addition of ALA to this combination, phenol oxidase activity decreased due to ALA. As a result, in this study, it was seen that ALA has a protective effect against the damage caused by MTX + ACT on rat lung tissue.

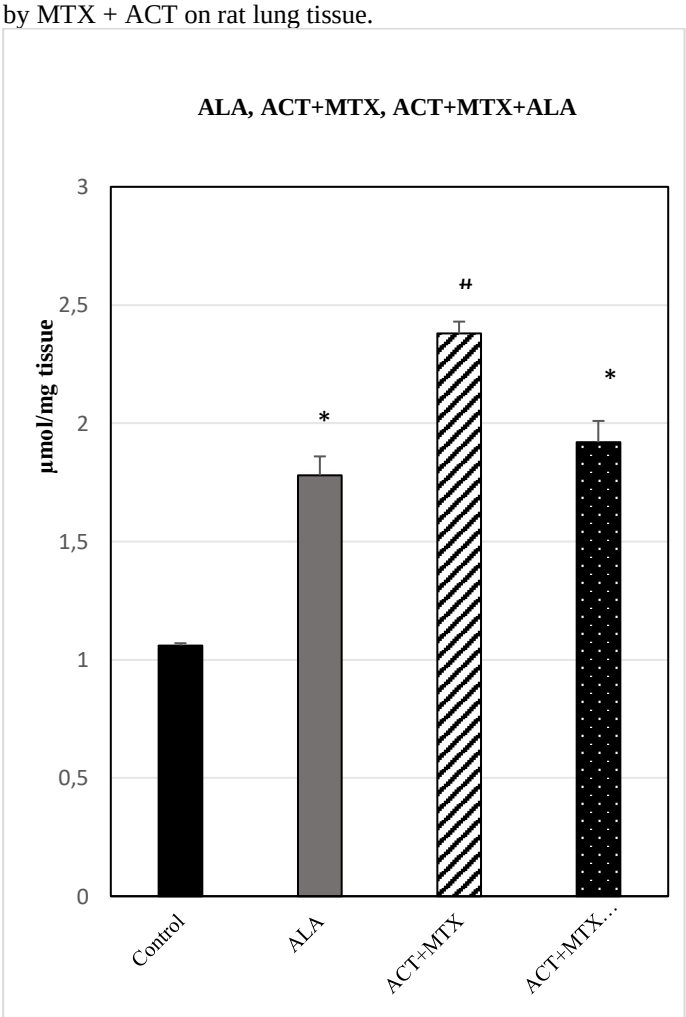

Fig. 4 Changes in Phenol Oxidase activity in the groups receiving ALA, MTX+ALA and MTX+ACT+ALA

(*$p<0.05$, ALA group compared to the control group), (#$p<0.05$, ACT+MTX group compared to the control group), (**$p<0.05$, ACT+MTX+ALA group compared to the control group)

5 Conclusion

In the study, it was observed that the combined use of ACT and MTX increased phenol oxidase activity in rat lung tissue. A decrease in this activity was observed with the addition of an antioxidant ALA to this combined application. When these results are evaluated, it can be said that MTX + ACT causes phenolic compounds in the lung tissue and ALA has a protective effect against MTX+ ACT derived phenolic compounds.

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