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Effects of antioxidant alfa-lipolic acid on polyphenol oxidase activity of acitretin-methotrexate combination in rat spleen tissue

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Abstract

Objectives: Acitretin, a lipophilic weak acid, is a slightly water-soluble vitamin A analog. Methotrexate is a folic acid analogue and an antimetabolite used for the treatment of many diseases.. Alpha lipoic acid is a nutritional coenzyme with important physiological effects in protein, fat and carbohydrate metabolism. Polyphenol oxidase is an oxidoreductase containing copper in its active site and oxidizes phenolic compounds. The aim of this study is to determine the effect of Alpha lipoic acid on the polyphenol oxidase enzyme activity in the spleen tissue in removing the damage caused by the free radicals produced by Acitretin and Methotrexate at the cellular level.

Materials and Methods: Study groups were formed as Control , Alpha lipoic acid , Acitretin+Methotrexate and Acitretin+Methotrexate+Alpha lipoic acid groups. Rats who were starved for 24 hours were injected at the every morning. Acitretin, Methotrexate and Alpha Lipoic Acid were dissolved in 0.9% NaCl and given to rats by intraperitoneal injection. Rats were sacrificed by cervical dislocation on the 7th day after injection. After perfusion of the heart, the spleen was removed. Extracted spleen tissue samples were used for polyphenol oxidase enzyme activity determination.

Results: Results: Compared with groups Control; While 18% inhibition was observed in the Alpha lipoic acid group, 20% inhibition was observed in the Acitretin + Methotrexate group. When Alpha lipoic acid was added to the Acitretin + Methotrexate group, while 19% activation was observed according to Control, 1% activation was observed compared to the Acitretin+Methotrexate group. Although there were differences between the groups, these differences were not statistically significant.

Conclusion: The inhibition of PPO enzyme activity by ALA can be explained by the fact that while ALA alone acts as a radical scavenger that can regenerate substances with antioxidant properties in the spleen tissue, when given in combination, it acts as an antioxidant that triggers PPO activity.

Keywords: Spleen; Acitretin; Methotrexate; Polyphenol Oxidase; PPO

1 Introduction

Acitretin (ACT), a vitamin A analog, is the active and predominant metabolite of etretinate. Because of this pharmacokinetics, its half-life is evaluated in the same way as etretinate[1-3]. Therefore, it has replaced etretinate in the treatment of keratinization disorders. In addition, ACT, a retinoid monoaromatic, has antiproliferative, anti-inflammatory, immunomodulatory and teratogenic properties [2,5,6,7,8,9]. Methotrexate (MTX, 4-amino-10-methylpteroylglutamic acid), a folic acid analogue used in the treatment of neoplastic or non-neoplastic diseases, inhibits the conversion of folic acid-regulating dehydrofolate (DHF) to tetrahydrofolate (THF).

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MTX has been reported to be used as an immunosuppressant in rheumatoid arthritis, inflammatory arthropathies, many types of cancer, and autoimmune diseases and also in organ transplant patients [12-15]. The organosulfur compound alpha-lipoic acid (ALA) can also be found in its reduced form as dihydrolipoic acid (DHLA). In its native form, it acts as a coenzyme for mitochondrial multienzyme complexes [16]. It has a strong antioxidant property with many functions such as chelating metals, scavenging reactive oxygen and nitrogen species, increasing the activities of free radical scavenging enzymes, contributing to the repair of damaged proteins and lipids [17,18]. Due to these properties, its protective effect against many diseases has been observed [19].Phenol oxidases (PPO; 1,2-benzenediol: oxygen oxidoreductase) containing Cu+2 in their active sites are metalloenzymes belonging to the class of oxido reductases. Physiological functions are mostly either pigmentation or protection from the harmful effects of the environment[20]. Having a very important role in the innate and acquired immune systems, the spleen plays critical supportive roles in the body's homeostasis by acting as a barrier and filter for blood[21,22]. However, no further data and reports on the spleen during oxidative stress are available.

2 Material and methods

In the study; *Wistar albino* male rats weighing 200 - 250 g, bred and raised at Ondokuz Mayıs University Experimental Animal Research Center (DEHAM), were used. This study was also approved by the Ondokuz Mayıs University Ethics Committee (2018/13). All rats were fed standard mouse chow. The rats were then divided into four groups: Control group (C), ALA group, ACT+MTX group, ACT+MTX+ALA group. There are five male mice per group. No treatment was applied to group C. To other groups, ALA (50 mg/kg/day) [23], ACT (20 mg/kg/day) [24] and MTX (20 mg/kg/week) [24] were given intraperitoneally at the same time every morning. At the end of the seventh day, all rats were sacrificed by cervical dislocation under anesthesia. As general anaesthesia, ketamine HCl (ketalar) (50 mg/kg) and Xylazine (Rompul) (10 mg/kg) were given. Then the heart was perfused with 0.9% NaCl and the spleens were removed. The spleens were homogenized first. Sonication and centrifugation were performed immediately after homogenization and the supernatant was obtained. Polyphenol oxidase (PPO) activity was determined in this supernatant[25]. This activity was determined by the method of Hung and Boucias (1996). For PPO activity; 50 µL of supernatant was added to a total volume of 1 ml to 20 mM L-DOPA phosphate buffer. Activity is the change in absorbance at 420 nm every 10 second intervals. An enzyme unit represents an increase of 0.001 in 1 minute in the cuvette where the reaction takes place.

2.1 Statistical analysis

SPSS 22.0 statistical program was used for statistical operations. Results are expressed as mean±. The standard error of the mean (statistical significance level) was accepted as $p<0.05$. Statistical differences between groups were analyzed using the non-parametric Kruskal-Wallis test due to non-normal distribution.

3 Results

When compared with the C group, while the ALA group was observed 18% inhibition, the ACT+MTX group was observed 20% inhibition ($p>0.05$) (Fig.1). When ALA was added to the ACT+MTX group, 19% inhibition ($p>0.05$) was observed compared to the K group, while 1% activation ($p>0.05$) was observed compared to the ACT+ MTX group (Fig. 2). Although the PPO activities of the groups were different among themselves and against the control group, these differences were not statistically significant (Table 1). When ALA is given alone, while it shows antioxidant properties by inhibiting PPO activity in the spleen tissue, it has been shown to trigger PPO activity when given with ACT+MTX combination.

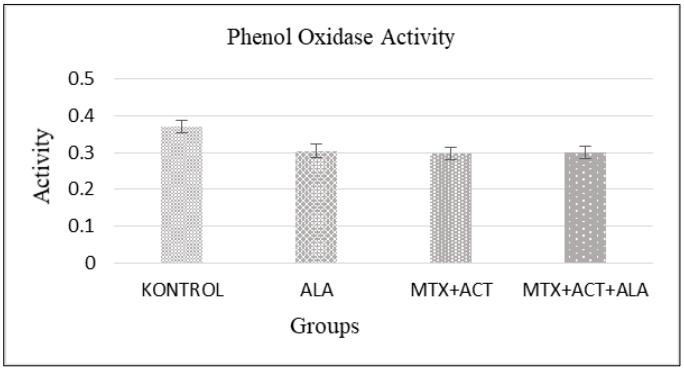

Figure 1 Intergroup comparison of PO activity in rat spleen tissue

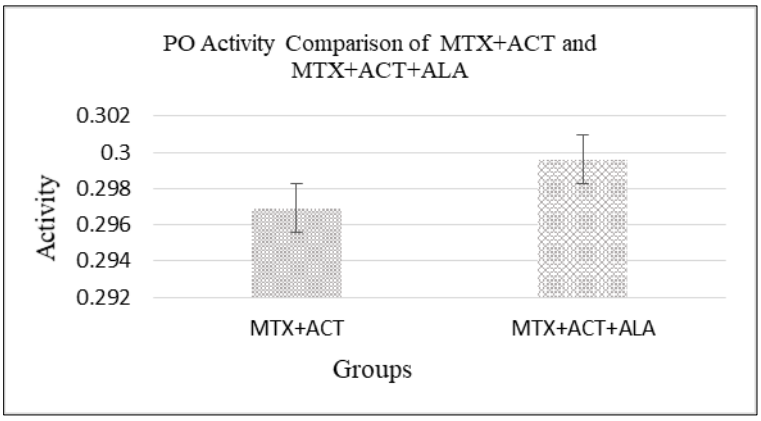

Figure 2 PO Activity Comparison of MTX+ACT and MTX+ACT+ALA in rat sleen tissue

Table 1 Statistical Analysis

	N	Mean	Std. Deviation	Std. Error	Sig.
ALA	5	0.3046400	0.05691949	0.02545517	0.331
MTX+ACT	5	0.2969000	0.03004813	0.01343793	0.247
MTX+ACT+ALA	4	0.2996000	0.04282180	0.02141090	0.301

4 Discussion

The spleen, one of the important organs of the immune system in the body, filters foreign compounds and acts as a pool for many blood cells[26]. There are almost no studies investigating the toxicity of MTX, especially on the spleen [27]. We investigated the therapeutic effects of ALA on PPO enzyme activity in repairing the damage caused by ACT and MTX in spleen tissue. Sezgin et al. (2022)[28] reported that the use of ACT+MTX caused PO inhibition in the rat brain on the third day, and this inhibition was then converted to activation. In the same study, it was observed that the inhibition observed on the third day with the effect of ALA decreased to half on the fifth and seventh days. In Shiga et al. (2020) [29] studies, MTX was applied sequentially instead of a single application. As a result, they observed that mucosal damage was exacerbated and significantly induced constitutive NOS expression in ileal tissue. Athoumani Ali et al. (2020) [30] showed that ACT and MTX increased PO activity in the lung, and when ALA was given with this combination, there was a decrease in activity.

Liu et al. (2021) [31], ACT was able to reduce spleen and skin lesions of imiquimod (IMQ)-induced psoriasis-like model mice; Bahceci et al., (2022) [32] reported that high-dose MTX administration increased T lymphocytes and macrophages in the spleen. In our study, it was observed that the combination of MTX + ACT caused inhibition of PO activity in the spleen. Yuan et al. (2022)[33] reported that polyphenol oxidase extracted from edible mushrooms significantly inhibited both proliferation and migration as well as invasion of some breast, lung, prostate cancer cell types. In addition, they reported that it significantly supported the apoptosis of 4T1, A549 and C4-2. They also concluded that edible mushroom extract polyphenol oxidase could play an effective role in the treatment of various cancers and potentially be a promising agent for the treatment of cancers.

It is known that the combined use of MTX and ACT has a curative effect in the treatment of psoriatic lesions[34]. It is also gaining importance that the spleen maintains its optimum function in terms of being the largest secondary immune organ[26]. Therefore, more studies are needed to reveal the effects of this combination and ALA on spleen tissue. According to the results of the study conducted by Yuan Q., et al. (2022) [33], we can say that it is also important to reveal the factors that change the PPO activity, considering the tumor growth inhibitory effect of the PPO enzyme. At this point, it is thought that the study will make an important contribution to the literature. According to the results of our study, the combination of MTX + ACT caused inhibition of PO activity in the spleen. Adding ALA to the MTX+ACT combination did not significantly change the effect of this combination. DHLA, the reduced form of ALA, can directly and indirectly regenerate ascorbate, glutathione, coenzyme Q10 and vitamin E [35].

5 Conclusion

In our study; The inhibition of PPO enzyme activity by ALA can be explained by the fact that while ALA alone acts as a radical scavenger that can regenerate substances with antioxidant properties in the spleen tissue, when given in combination, it acts as an antioxidant that triggers PPO activity. Conducting new studies by creating differences in exposure times and doses to active substances will contribute to the elucidation of the mechanism.

Compliance with ethical standards

Acknowledgments

This study was found to be in compliance with the Animal Rights and Experimental Ethical Principles by the Animal Experiments Local Ethics Committee of Ondokuz Mayıs University (OMU-HADYEK).

Disclosure of conflict of interest

There is no conflict of interest between the authors.

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