RESEARCH ARTICLE
Bull Biotechnol (2020) 1(1):8-12
Jurnal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Effect of different combinations of alpha lipoc acid, acitretin and methotrexate on malondialdehyde levels in the kidney tissues of rats

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Received : 04/05/2020
Accepted : 02/06/2020

Abstract: Acitretin (ACT), which is a second generation retinoid, exerts a therapeutic effect. It also has an anti-inflammatory and antiproliferative effect. It can also be used alone or in combination in the treatment of some diseases. In the use of methotrexate (MTX) alone, it can cause serious side effects such as hepotoxicity and nephrotoxicity. As a result of its use with ACT in recent years, it has been shown to have side effects on some organs as well as being beneficial. In this study, the effects of MTX, ACT and ALA on malondialdehyde (MDA) levels in rat kidney tissue were investigated. For this purpose, rats were given ALA, ACT + MTX and ACT + MTX + ALA by intraperitoneal injection. Following this, after the injections on the 3rd, 5th and 7th days, the kidneys were removed by applying cervical dislocation to the rats. The kidneys were homogenized, sonified and centrifuged. The fractions obtained as a result of these processes were used to investigate the effects on MDA levels. As a result, when compared to the MDA levels in the control group, an overall increase was observed in the group given MTX + ACT. It was determined that the increase continued with the administration of ALA with MTX + ACT. Only in the group where ALA was given, a decrease in MDA level was observed.

Keywords: Malondialdehyde; acitretin; methotrexate; alpha lipoic acid; kidney

1 Introduction

Acitretin (ACT), which is a seond generation retinoid monoaromatic, is a vitamin A analog (Khalil et al. 2017). It is also a retinoic acid metabolite of etretinate and is preferred due to its pharmacokinetic advantage (Özarmağan, 2016). ACT has antiproliferative, anti-inflammatory and immunomodulating properties (Tu et al. 2020). Besides being used in the treatment of diseases such as Hidradenitis suppurativa, Psoriasis, Pityriasis rubra pilaris, Lichen planus, Ichthyosis, Darier's disease, Actinic keratosis (Khalil et al. 2017), it also has a teratogenic effect since it is a retinoid (Özarmağan, 2016).

Methotrexate (MTX) belongs to an antineoplastic drug group, and is an analog of folic acid (Shingirik et al. 2019). It inhibits the conversion of dehydrofolate (DHF), which is necessary for the regulation of folic acid, to tetrahydrofolate (THF). Thus, it causes THF deficiency, which plays a role in the synthesis of purine and pyrimidine nucleotides, and causes disruptions in protein metabolism with nucleic acid (Güven et al. 2017). It is used for therapeutic purposes in diseases such as psyroatic and rheumatoid arthritis, psoriasis and ectopic pregnancy (Alinejad et al. 2019). At the same time, MTX is used in lung, breast, acute lymphoblastic leukemia, lymphoma, and head and neck cancers because it inhibits cell division (Güven et al. 2017). In addition to the therapeutic properties of MTX, toxic effects can also be seen. This toxicity occurs as a result of oxidative stress caused by free radicals (Ali et al. 2017). As a result of MTX-induced toxicity, the risk of intestinal-related sepsis, interstitial pneumonia in the lungs, and bone marrow suppression can be observed (Chabner et al. 2007). In addition, hepatoxicity and nephrotoxicity are also serious side effects of MTX (Şentürk, 2016). MTX, which causes toxicity even when used alone, has also been shown to have side effects in combination use. It is recommended to be careful in drug combinations against these side effects and to give an antioxidant together with combination use when necessary (Armağan, 2015).

Alpha lipoic acid **(**ALA) is an important naturally produced antioxidant that acts as a cofactor in mitochondial enzymes, found in most prokaryotic and eukaryotic cell types (Cadenas, 2001). ALA may have duties such as regeneration of vitamins C and E, the possibility of lipid peroxidation and protection against free radicals, increasing superoxide dismutase and catalase enzyme activities, repairing molecular damage and increasing acetylcholine production (Tetikçok et al. 2015). It has been observed that it regulates blood glucose in patients with Type 1 and Type 2 *Diabetes Mellitus* (Khamaisi et al. 1997) and helps healing in some diabetic retinopathic (Kilic et al. 1998) and neuropathic patiens (Ziegler et al. 1999). In addition, it has been observed that it has positive effects on cognitive functions in some neurological diseases (Cui et al. 2006). In addition to these, it also shows a protective effect against some

cardiovascular, neurological and diseases caused by viral infections (Özgün et al. 2018).

In light of this information, in recent years, although the side effects are known, the use of ACT + MTX combination has increased in the treatment (An et al. 2017). Therefore, in this study, the effect of ACT-MTX combination on malondialdehyde (MDA), which is a marker of cell damage in rat kidney tissue, and whether it has protective role of antioxidant ALA was investigated.

2 Materials and Method

2.1 Experimental Animals

In this experimental study, 50 Wistar albino type male rats (weighing between 250 and 300 g) were used all animals were obtained from the Ondokuz Mayıs University, Experimental Animals Application and Research Center (DEHAM), which was maintained for 12 hours in light and 12 hours in the dark and at a temperature of 21° C. 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 free.

2.2 Formation of Research Groups

The study groups were formed as the Control group (C), the ALA group, the ACT + MTX group and the ACT + MTX + ALA group. There are 15 rats in each of ALA, ACT + MTX, ACT + MTX + ALA groups that make up the experimental groups.

2.3 Injection and Obtaining Kidneys

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) (An 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. Rats in the experimental groups were sacrificed by cervical dislocation method by giving general anesthesia on the 3rd, 5th and 7th days after the injection. 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 kidneys were removed. These kidneys were placed in containers containing isotonic sucrose and stored in a freezer at -80 ° C for experimental procedures.

2.4 Preparation of Kidney Tissues for Analysis

After the tissues 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 stored at -80 ° C.

2.5 MDA Measurement in the Kidney Homogenates

MDA levels in kidney homogenates were measured by double warming, as described by Draper and Hadley. The principle of this method is the spectrophotometric measurement (at 532 nm) of the end product of the lipid peroxidation reaction with thiobarbituric acid (Draper and Hadley, 1990). An important marker of lipid peroxidation, MDA reacts with thiobarbituric acid (TBA) and forms a pink complex. The pink colored complex formation produced by MDA and thiobarbituric acid (TBA) reaction was evaluated by spectrophotometric measurement (at 532 nm). The MDA concentration was calculated with a calibration curve expressed as μmol / mg protein .

2.6 Statistics

All data were evaluated using SPSS 22.0 statistical package program. Since the groups did not show normal distribution, it was checked with Kruskal Wallis test whether there would be a difference between C, ALA, ACT + MTX and ACT + MTX + ALA groups. Values with $p < 0.05$ were considered significant.

3 Results

When the group given ALA is compared with the C group; On the 3rd day following the injection, a decrease of approximately 31% in MDA level was observed. This decrease was 28 % on the 5th day and 18% on the 7th day (Fig 1).

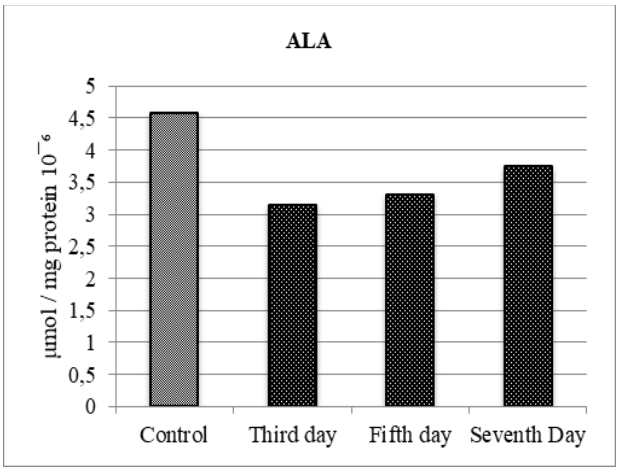

Fig.1 The levels of MDA in the ALA group

In the ACT + MTX group, the MDA level increased by 2 % on the 3rd day compared to the control. It was observed that the increase continued to increase on the 5th and 7th days and was approximately 10 % and 17 %, respectively (Fig 2). The ACT + MTX + ALA group increased by 98 % on the 3rd day compared to the control group. This increase, which was 16 % on the 5th day, was 31% compared to the control on the 7th day (Fig 3).

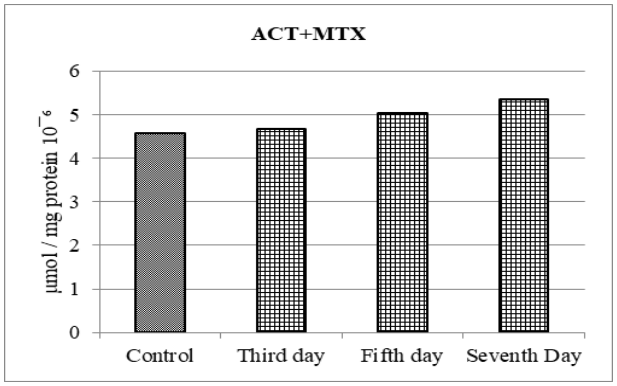

Fig. 2 The levels of MDA in the ACT+MTX

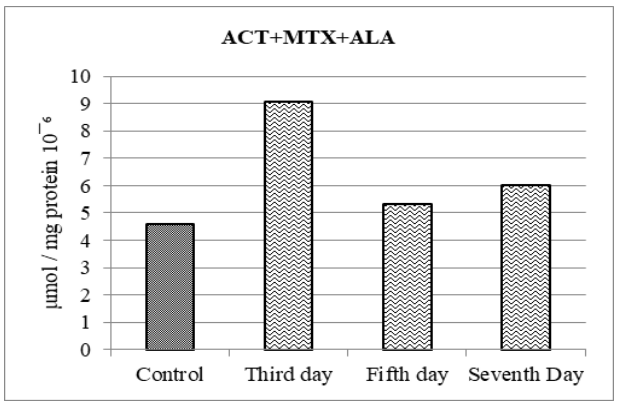

Fig. 3 The levels of MDA in the ACT+MTX+ALA

When we look at our work in general; compared to the C group, MDA levels decreased (p> 0.05) in the group given ALA and increased in the group given ACT+MTX (p>0.05). With the addition of ALA to the ACT+MTX combination , an increase in MDA levels was observed (p < 0,05) (Fig 4).

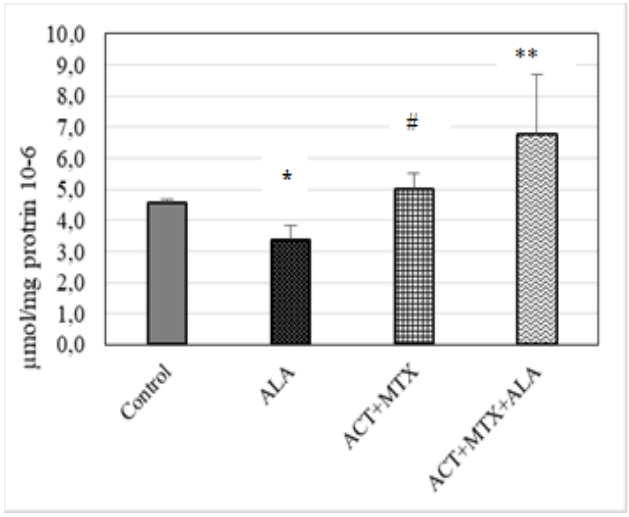

Fig. 4 The levels of MDA in the ALA, ACT+MTX and ACT+MTX+ALA groups

(*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)

4 Discussion

This study; It was made to investigate the effect that will occur as a result of the addition of an antioxidant ALA to the changes on MDA levels, which is an oxidative stress marker in rat kidney tissues that have applied ACT and MTX.

ACT is the secondary generation among systemic retinoids and is used for therapeutic purposes, especially in the field of dermatology (Khalil et al. 2017). However, in its use alone; Side effects such as an increase in transminase, triglyceride and cholesterol values and a decrease in HDL can be seen. Although these side effects are dose and time dependent, caution should be exercised and regular follow-up should be taken during the drug intake due to its teratogenic effect. In combination treatment, side effects and various risks can be seen while increasing effectiveness (Özarmağan, 2016).

MTX provides suppression of dermal inflammatory infiltration and prevention of cell proliferation. It is also used in the treatment of diseases in the field of dermatology and cancer. Also (Şentürk, 2016) in the compilation study; In addition to its therapeutic effect, it emphasized the necessity to pay particular attention to it single use and combined use as it has toxic properties on various tissues and organs such as hepatoxicity and nephrotoxicity. She also stated that dosage and duration adjustment should be done well and careful monitoring of patients against toxicity.

ALA can prevent the formation of reactive oxygen species as it forms chelates with metals. Thus, it has been determined that it can reduce mortality by using it as a treatment in some metal poisoning. In addition, it has been stated that it is a powerful antioxidant that can be used in the treatment of various diseases in order to stop and prevent the progression of tissues as a result of oxidative stress (Bludovska et al. 1999; Gomes and Negrato, 2014). In addition, no excessive side effects were found unless there was an overdose. It can be used to reduce and prevent the toxicity that may occur in combination therapies and the treatment of toxic drugs (Yürük and Ayaz, 2014; Ergene, 2018).

In one study, it has been concluded that ACT and MTX combination therapy are not faced with a new or extraordinary condition, including hepatitis or liver toxicity. And it has been reported that it can be used as a combination of ACT and MTX at low doses. It has also been stated that simultaneous use of MTX will not be an absolute contraindication in patients who will benefit from the use of ACT (Lowenthal et al. 2008).

An et al. (2017), when ACT and MTX combination groups are compared with MTX used groups they found that the increase in profibrotic factors in serum was less. In addition, in this study, they thought that ACT and MTX combination may provide higher efficacy in the treatment of psoriasis and decrease in liver fibrosis. As a result, it is stated that if appropriate doses are used, ACT and MTX can be used as a combination.

In our study, it was observed that in the groups in which ACT + MTX was given, it caused an increase in MDA level. This increase in MDA level is also known as a marker of damage to kidney tissue. It is thought that the reason for this situation may be due to the toxic effect of MTX.

In the study of Armağan et al. (2015), it was observed that there was an increase in MDA level in kidney tissu es of MTX group compared to C. It was observed that this increase decreased in the treatment group that received MTX + ALA. It has also been concluded that ALA is effective with antioxidant and other properties in preventing the toxic effect of MTX in kidney tissue.

In another study, the effects of luteolin (LUT) with strong antioxidant and anti-inflammatory properties against MTX-induced liver toxicity in rats were investigated. As a result of the researches, an increase in MDA level was observed in the groups that received MTX in liver tissue. It was observed that this increase decreased in MTX and LUT applied groups. As a result of the biochemical evaluations, it was observed that while MDA values increased in the group receiving MTX in liver tissue, it decreased in the LUT group. It has been concluded that LUT treatment reduces MTX-induced damage (Gedik, 2019).

In our study, while the increase of MDA level was observed in the group given ACT + MTX, it was also determined that it did not decrease in the ACT + MTX + ALA experimental group. Thus, it has been observed that ALA, which is used as an antioxidant, causes an increase in MDA in all the days it is added to the combination. However, when using ALA alone, it was seen that there was a decrease in MDA levels. And it has been determined that ALA does not have a condition that causes tissue damage when used alone.

Aksoy (2017) investigated the effect of ALA on testicle damage caused by busulfan (BUS) in his study. As a result of the study; Compared with the C group, it was observed that the MDA level was high in the group where BUS was given, it was less in the group that received ALA, and there was an increase in the group that received BUS + ALA. As a result, it was observed that BUS caused damage to the testicles, but it did not show its protective and / or therapeutic effect in the use of ALA with BUS.

When our results are compared with the literatüre, it was found to be in agreement with the results in testicular tissue while in contrast with the results in liver and kidney tissue.

5 Conclusion

As a result, it was observed that the combined use of ACT and MTX caused damage to rat kidney tissue. With the addition of an antioxidant ALA to this combination application, while the damage is expected to decrease, an increase in the indicator MDA has been observed. Thus, in this study, it was concluded that ALA did not show a therapeutic effect in use with ACT and MTX for kidney tissue.

According to our data , it was thought that a study to be conducted using an increase in the number of days and using different doses could contribute to the literature.

Acknowledgements

This work was supported by Ondokuz Mayıs University, BAP with the project numbered PYO.FEN.1904.19.009.

Authors' contributions: B.B.,E.D.and F.G.S.designed and performed the experiment. They also analyzed the data and wrote the manuscript.

Conflict of interest disclosure:

We have no conflict of interest with any people or organization.

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