Effects of Alpha-Lipoic Acid on Polyphenol Oxidase Activity in Small Intestine Tissue of Rats Treated with Acitretin - Methotrexate Combination

Fatma Gönül SEZGİN[1], Emine İncilay TORUNOĞLU[2], Emine DIRAMAN[3]*

Research Article/AraştırmaMakalesi

Geliş Tarihi / Received: *19.12.2022*
Kabul Tarihi / Accepted: *29.12.2022*

ABSTRACT

Background and Aim: Acitretin is a vitamin A analogue, which is a lipophilic weak acid, less soluble in water and less accumulated in adipose tissue. It is also non-immunosuppressive with anti-inflammatory and anti-proliferative effects. Methotrexate, an inhibitor of thymidylate synthetase and dihydrofolate reductase, is a folic acid analogue. Today, Methotrexate, which is used in the treatment of diseases such as mycosis fungoides, pityriasis rubra pilaris, dermatomyositis, sarcoidosis, eczema, as well as cancer treatments, is also an anti-folate antimetabolite agent. It has also been used together with Acitretin in treatment. In this study, Alpha Lipoic Acid, which is a powerful antioxidant, was given to rats to eliminate the damage caused by free radicals that may occur as a result of Acitretin-Methotrexate application. Thus, it is aimed to investigate the effect of Alpha Lipoic Acid, which plays an important role in repairing the oxidative damage that may occur, on the polyphenol oxidase (1.10.3.1) enzyme activity in the small intestine tissue. Polyphenol oxidase, containing copper in its active site, is an oxidoreductase class enzyme that catalyzes the oxidation of phenolic compounds with molecular oxygen.

Materials and Methods: Four study groups were formed as Control Group, Alpha Lipoic Acid Group, Acitretin+Methotrexate Group and Acitretin+Methotrexate+Alpha Lipoic Acid Group. The injection procedures applied to the rats were performed at the same time every morning and they were fasted 24 hours before the injection. 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. Following this, cardiac perfusion was performed and the small intestines were removed. The samples were first homogenized, then sonication and centrifugation processes were applied. After centrifugation, the obtained small intestine homogenates were used for Polyphenol oxidase activity determination.

Result and Conclusion: Alpha Lipoic Acid, Acitretin + Methotrexate and Acitretin + Methotrexate + Alpha Lipoic Acid groups were compared with the control group. As a result, while it was observed that the Alpha Lipoic Acid group had almost the same activity with the Control group (1.65% activation), the Acitretin + Methotrexate group showed 10% activation, the Acitretin + Methotrexate + Alpha Lipoic Acid group showed 25% activation. When the Acitretin + Methotrexate group was compared with the Acitretin + Methotrexate + Alpha Lipoic Acid group, it was also determined that the Acitretin + Methotrexate + Alpha Lipoic Acid group showed 13.5% more activation. While Alpha Lipoic Acid did not show a significant difference in Polyphenol oxidase activity in small intestine tissue when given alone, it increased Polyphenol oxidase activity when given with Acitretin + Methotrexate combination.

Keywords: Small intestine, acitretin, methotrexate, polyphenol oxidase

[1] PhD, Res. Asst. Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology, Samsun/Turkey, email: gonul.solmaz@omu.edu.tr, ORCID ID: 0000-0002-9400-5173

[2] PhD student, Necmettin Erbakan University, Faculty of Medicine, Department of Biochemistry, Samsun/Turkey, email: torunogluemineincilay@gmail.com, ORCID ID: 0000-0003-4641-0067

[3] Assoc. Prof. Dr., Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology, Samsun/Turkey, email: ediraman@omu.edu.tr, ORCID ID: 0000-00002-4677-1738

***Corresponding author**

Asitretin-Metotreksat Uygulanmış Ratların İnce Bağırsak Dokusunda Polifenol Oksidaz Aktivitesi Üzerine Alfa-Lipoik Asitin Etkileri

ÖZET

Amaç: Asitretin, lipofilik zayıf bir asit olan suda az çözünen ve yağ dokusunda az biriken A vitamini analoğudur. Aynı zamanda anti-inflamatuar ve anti-proliferatif etkili non-immünosupresifdir. Timidilat sentetaz ve dihidrofolat redüktaz inhibitörü olan metotreksat ise bir folik asit analoğudur. Günümüzde de kanser tedavilerinin yanında mikozis fungoides, pitriyazis rubra pilaris, dermatomiyozit, sarkoidoz, egzama gibi hastalıkların tedavisinde de kullanılan Metotreksat aynı zamanda bir anti-folat antimetabolit ajandır. Ayrıca Asitretin ile kombinasyon halinde tedavide de kullanılmaya başlanmıştır. Bu çalışmada, Asitretin-Metotreksat uygulaması sonucunda meydana gelebilecek serbest radikallerin neden olduğu hasarın giderilmesinde güçlü bir antioksidan olan Alfa Lipoik Asit ratlara verilmiştir. Böylece oluşabilecek oksidatif hasarı onarma da önemli rol oynayan Alfa Lipoik Asitin ince barsak dokusundaki polifenol oksidaz (1.10.3.1) enzim aktivitesi üzerine etkisinin araştırılması amaçlanmıştır. Aktif bölgelesinde bakır içeren Polifenol oksidaz, fenolik bileşiklerin moleküler oksijen ile oksidasyonunu katalizleyen oksidoredüktaz sınıfı bir enzimdir.

Yöntem: Kontrol grubu, Alfa Lipoik Asit grubu, Asitretin+Metotreksat grubu ve Asitretin+Metotreksat+Alfa Lipoik Asit grubu olarak dört çalışma grubu oluşturulmuştur. Ratlara enjeksiyon işlemleri her sabah aynı saatte yapılmıştır ve enjeksiyondan 24 saat önce de aç bırakılmışlardır. Asitretin, Metotreksat ve Alfa Lipoik Asit % 0.9'luk NaCl'de çözülmüştür ve intraperitonal enjeksiyonla ratlara verilmiştir. Ratlar, enjeksiyondan sonraki 7. günde servikal dislokasyon ile sakrifiye edilmiştir. Bunu takiben kalp perfüzyonu işlemi yapılmış ve ince bağırsakları çıkarılmıştır. Örnekler önce homojenize edildi sonra sonikasyon ve santrifüj işlemleri uygulandı. Santrifüj işleminden sonra, elde edilen ince bağırsak homojenatları Polifenol oksidaz aktivite tayini için kullanılmıştır.

Bulgular ve Sonuç: Alfa Lipoik Asit, Asitretin + Metotreksat ve Asitretin + Metotreksat + Alfa Lipoik Asit grupları kontrol grubu ile karşılaştırılmıştır. Bunun sonucunda Alfa Lipoik Asit grubunun Kontrol grubu ile hemen hemen aynı aktiviteye sahip olduğu (%1,65 aktivasyon) gözlenirken Asitretin + Metotreksat grubu %10, Asitretin + Metotreksat + Alfa Lipoik Asit grubunun ise %25 aktivasyon gösterdiği gözlenmiştir. Asitretin + Metotreksat grubu ile Asitretin + Metotreksat + Alfa Lipoik Asit grubu karşılaştırıldığında Asitretin + Metotreksat + Alfa Lipoik Asit grubunun %13,5 daha fazla aktivasyon gösterdiği de belirlenmiştir. Alfa Lipoik Asit, tek başına verildiğinde ince bağırsak dokusunda Polifenol oksidaz aktivitesinde anlamlı bir fark göstermezken, Asitretin + Metotreksat kombinasyonu ile verildiğinde Polifenol oksidaz aktivitesini artırmıştır.

Anahtar Kelimeler: İnce bağırsak, asitretin, metotreksat, polifenol oksidaz

INTRODUCTION

Polyphenols, which are compounds containing the phenol group, have antioxidant properties. Therefore, they show the ability to scavenge various reactive oxygen species such as free oxygen, peroxynitrite, hydrogen peroxide. Phenol oxidases (PPO; 1,2-benzenediol: oxygen oxidoreductase) are metalloenzymes belonging to the class of oxido reductases containing Cu+2 in their active sites. These enzymes catalyze two activities in the presence of O2. One of the activities is the hydroxylation of o-monophenols to o-diphenols (cresolase activity), and the other is the oxidation of o-diphenols to o-quinones (catecholase activity). (Steffens et al., 1994; Rudrapal et al., 2022).

Acitretin (ACT) is a retinoid (Fig.1), (Ortiz et al., 2013). Retinoids, which have activity similar to vitamin A, are natural and synthetic compounds. Among the effects of retinoids; There are also effects such as immunological anti-inflammatory effects, induction of apoptosis and inhibition of tumor growth (Mehrtens et al., 2018; Ighani et al., 2019; Skillen et al., 2019). ACT, which is a lipophilic weak acid, is an analogue of vitamin A, which is less water-soluble and less accumulated in adipose tissue, and is also a non-immunosuppressive with anti-inflammatory and anti-proliferative effects (Zito and Mazzoni, 2022).

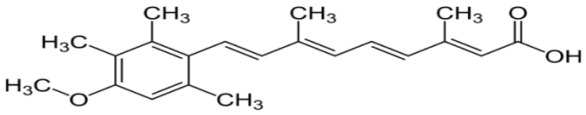

Fig.1. Chemical structure of ACT (Bhuiyan and Chowdhury, 2016)

ACT is used in the treatment of psoriasis and keratinization disorders. It has also been used for the treatment of many dermatoses such as hyperkeratotic and inflammatory dermatoses, non-melanoma skin cancers. Since it is both a potent teratogen and embryotoxic, it has also been reported to be contraindicated in women who are pregnant and have high potential for pregnancy. It is also used in the treatment of Alzheimer's (Holthoewer et al., 2012; Davis et al., 2013; Guenter et.al., 2017; Sadowska et al., 2022).

Methotrexate (MTX; 4-amino-10-methylpteroylglutamic acid), a folic acid analogue, is also an inhibitor of thymidylate synthetase and dihydrofolate reductase enzymes (Fig.2), (Lagarce et al., 2015). In today's world, MTX; In addition to being one of the chemotherapeutic options for many cancer types, it is also used as an immunosuppressant in autoimmune diseases and in the treatment of neoplastic diseases (Braun and Rau 2009; Hannoodee and Mittal 2022). It has also been reported to be used in patients with organ transplantation, as it has anti-inflammatory and immunomodulatory activities (Chan and Cronstein, 2010).

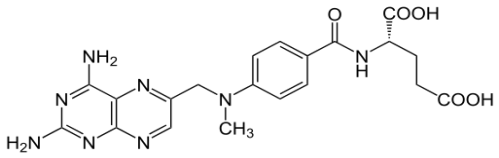

Fig.2. Chemical structure of Methotrexate (Lotfi et al., 2016)

In addition to being effective in ulcerative colitis, lymphoma (non-Hodgkin type), head and neck epidermal tumor and treatment, it is also used in breast, small cell lung and ovarian carcinomas (Chande et al., 2014). It is used in the treatment of diseases such as psoriasis, eczema, systemic lupus erythematosus, vasculitis, mycosis fungoides, dermatomyositis, pityriasis rubra pilaris, inflammatory bowel disease, sarcoidosis, and non-metastatic osteosarcoma (Bedoui et al., 2019; Hannoodee and Mittal, 2022). The low-dose use of MTX was first proposed for the treatment of rheumatoid arthritis (Weinblatt et al., 1985).

Fat and water soluble alpha-lipoic acid (ALA) is effective in preventing lipid peroxidation and scavenging free radicals with its strong antioxidant properties (Fig.3). ALA is required for the oxidative decarboxylation of pyruvate to acetyl-CoA, which bridges the gap between glycolysis and the citric acid cycle. It also acts as a cofactor in pyruvate dehydrogenase and α-keto-glutarate dehydrogenase activity in the citric acid cycle (Schmidt et al., 1994; Reed, 1998). Since it has the ability to cross the blood-brain barrier, it can act as a potential brain antioxidant and a therapeutic agent (Shay et al., 2009, Molz and Schröder, 2017). It has the effect of increasing the activities of radical scavenging enzymes and plays an important role as a cofactor that helps enzymatic nutrient breakdown in protein, fat and carbohydrate metabolism (Data 1995; Packer et al., 2011). It plays a role in the treatment of diabetic neuropathy by reducing lipid peroxidation in the nervous tissue (Kahler et al., 1993; Vallianou et al., 2009; Golbidi et al., 2011).

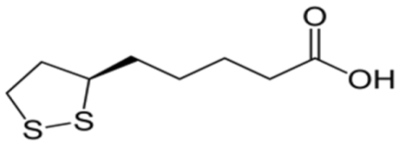

Fig. 3. Chemical structure of Alpha-Lipoic Acid (Molz and Schröder, 2017)

METHODS

This study was approved by the Ondokuz Mayıs University Ethics Committee (2018/13). Wistar albino type male rats weighing between 200 and 250 grams were used and these rats were obtained from Ondokuz Mayıs University, Experimental Animal Research Center (DEHAM). They were fed with standard mouse food and water was given freely.

Working groups:

C Group : Control group, no action was taken.

ALA Group: The group given 50 mg/kg/day ALA,

ACT + MTX Group: The group given 20 mg /kg /day ACT and 20 mg /kg /week)MTX,

ACT + MTX + ALA Group: The group given 20 mg /kg /day ACT, 20 mg /kg /day MTX and 50 mg / kg /day ALA

In each of the groups five rats were used. After starving for 24 hours, the rats were injected 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 combinations were given to the rats as an intraperitoneal injection (i.p.) at the body weight dose of each. On the 7th day after the injection, the rats were sacrificed under general anesthesia. As general anesthesia, 50 mg/kg ketamine HCl (ketalar) and 10 mg/kg Xylazine (Rompul) were given. Next, the heart was perfused with 0.9% NaCl and the small intestines were removed. Stored at -80°C. Afterwards, the samples removed from the deep freezer were first homogenized, then sonication and centrifugation processes were applied. After centrifugation, the obtained small intestine homogenates were used for PPO activity determination. This activity determination was made according to the method of Hung and Boucias (1996).

For PPO activity; 50 µL of homogenate was rapidly added to 950 µL of phosphate buffer solution containing 20 mM L-DOPA. Activity was determined by reading the change in absorbance at 420 nm at 10 second intervals per minute. As a result; one enzyme unit is defined as an increase of 0.001 per 1 minute in the cuvette where the reaction occurs.

Statistical analysis

All data were evaluated using SPSS 26.0 statistical software. Since the data were not homogeneously distributed in the One-Way Anova test, the Games-Howell multiple comparison test was applied.

RESULT

In our study, when compared with the C group, it was observed that the ALA group had almost the same activity as the C group (1.65% activation), while the ACT+MTX group showed 10% activation compared to the C group. It was observed that the ACT+MTX+ALA group showed 25% activation compared to the C group. When the ACT+MTX group and the ACT+MTX+ALA group were compared, it was observed that the ACT+MTX+ALA group showed 13.5% activation compared to the ACT+MTX group (Fig.4; Fig.5).

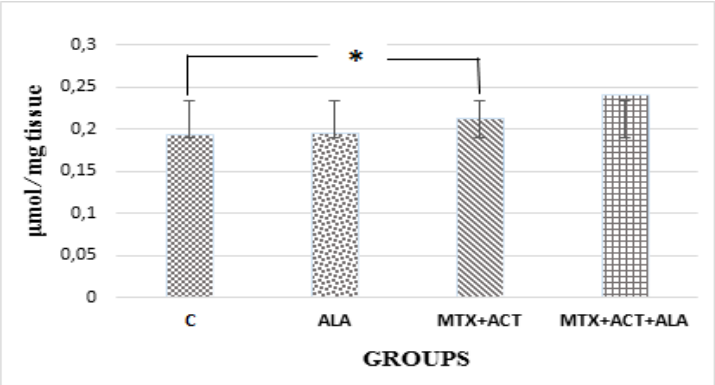

Fig.4. Changes in PPO activity in the groups receiving ALA, MTX+ALA and MTX+ACT+ALA

(* The mean difference is significant at the 0.05 level)

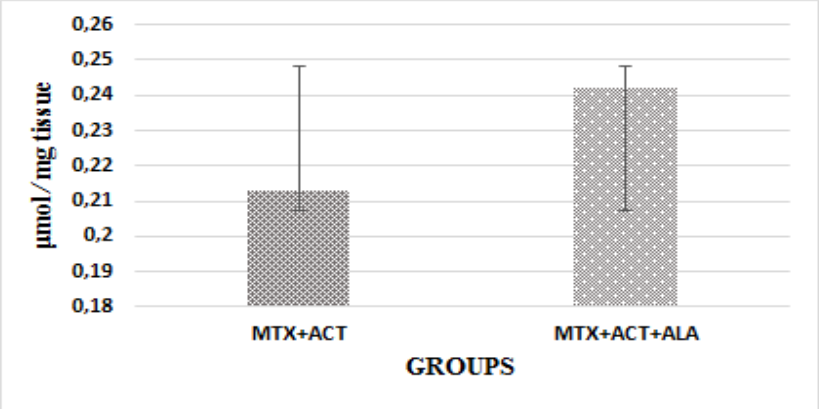

Fig.5. Comparison of PPO activity between ACT+MTX and ACT+MTX+ALA groups

Table 1. Multiple Comparisons Games-Howell Test

		Mean Difference	Std. Error	Sig.
CONTROL	ALA	-,0029200	,0039449	,877
	MTX+ACT	-,0193600*	,0048754	,038
	MTX+ACT+ALA	-,0483333	,0175929	,253
ALA	CONTROL	,0029200	,0039449	,877
	MTX+ACT	-,0164400	,0054454	,069
	MTX+ACT+ALA	-,0454133	,0177593	,277
MTX+ACT	CONTROL	,0193600*	,0048754	,038
	ALA	,0164400	,0054454	,069
	MTX+ACT+ALA	-,0289733	,0179889	,512
MTX+ACT+ALA	CONTROL	,0483333	,0175929	,253
	ALA	,0454133	,0177593	,277
	MTX+ACT	,0289733	,0179889	,512

*. The mean difference is significant at the 0.05 level.

DISCUSSION AND CONCLUSION

In this study, it was aimed to investigate the effect of ALA, which is a powerful antioxidant in the elimination of damage caused by free radicals formed by ACT and MTX, and plays an important role in repairing oxidative damage, on the activity of PPO enzyme in the small intestine tissue. PPO is an enzyme of the oxidoreductase class, which contains copper in its active sites, catalyzes the oxidation of phenolic compounds with molecular oxygen.

Wang and Tsai (2014) reported that ACT was used for 25 months for the treatment of a male individual with Darier's disease, and as a result, small intestine perforation developed as a result of severe inflammation of the small intestine of the male individual. An et al. (2017) found that when ACT and MTX are given together, they cause toxicity in the liver and the addition of ALA to this combination reduces hepatotoxicity. It has also been shown that oxidative stress caused by MTX is reduced by ALA (Arpag et al., 2018). In another study, while it was observed that administration of ACT and MTX together increased PPO activity in the lung tissue, administration of ALA decreased this activity (Athoumani et al., 2020). The results of Shiga et al.'s (2020) studies showed that sequential application of methotrexate rather than a single application exacerbated mucosal damage and significantly induced constitutive NOS expression in ileal tissue. In addition, based on these results, they stated that sequential administration of methotrexate rather than a single application also exacerbates mucosal damage. In another study, a case of inflammatory colitis due to MTX toxicity in a patient with psoriasis was presented. In the known side effects of MTX; It should be kept in mind that there is bone marrow suppression and inflammation in the mucous membranes and follow-up is recommended (Kilinc et al., 2021). MTX is a drug frequently used in the treatment of rheumatoid arthritis (RA) as it is used in other diseases, but lymphoproliferative disorders have been reported to occur in patients on the MTX regimen. When such a situation is encountered, discontinuation of methotrexate and small intestine resection have been recommended (Nomura et al., 2021). In the studies of Sezgin et al. (2022), it was determined that the combination of ACT and MTX increased the activity of PPO in the rat brain tissue, and there was a further increase in the activity with the addition of ALA to this combination.

In our study, when compared with the C group, it was observed that the ALA group had almost the same activity as the C (1.65% activation). It was determined that the combination of ACT+MTX increased PPO activity in the small intestine. Considering that ACT is an analog of vitamin A, we can say that it shows an antioxidant feature that triggers PPO activity thanks to this structure. While ALA alone did not show a significant effect in the small intestine, when added to the ACT+MTX combination, it showed an effect that further increased PPO activity. This is also supported when the MTX+ACT group is compared with the MTX+ACT+ALA group. While MTX+ACT showed 10% activation according to C, it was determined that it showed 25% activation with the addition of ALA to this group.

In the literature, while long-term use of ACT, MTX has been shown to cause damage to the

small intestine, according to the results of our study, the combination of ACT+MTX showed an effect that increased PPO activity in the small intestine. It was observed that the activation was further increased with the addition of ALA to the ACT+MTX combination. On the other hand, it was concluded that there was no significant difference in PPO activity when ALA was given alone.

In order to explain the behavior of PPO activity against these substances in detail, new studies are recommended in which exposure time differences are created and different doses of active substances are applied.

Conflict of Interests

The authors declare that they have no conflict of interest.

REFERENCES

An, J., Zhang, D., Wu, J., Li, J., Teng X, Gao X, Xia Y. (2017). The acitretin and methotrexate combination therapy for psoriasis vulgaris achieves higher effectiveness and less liver fibrosis. *Pharmacol Res. 121*, 58-168.

Arpag, H., Gül, M., Aydemir, Y., Atilla, N., Yiğitcan, B., Cakir, T., Polat, C., Þehirli, Ö., Sayan, M. (2018). Protective effects of alpha lipoic acid on methotrexate-ınduced oxidative lung injury in rats. *J. Invest. Surg. 31*(2):107-113

Athoumani Ali, S., Dıraman, E., Solmaz, F., G. (2020). The investigation of the effect of alpha lipoic acid on lung polyphenol oxidase activity in acitretin and methotrexate given rats. *Bull Biotechnol.,1*(1):19-22

Bedoui, Y., Guillot, X., Sélambarom, J., Guiraud, P., Giry, C., Jaffar-Bandjee, M. C., Ralandison S., Gasque, P. (2019). Methotrexate an Old Drug with New Tricks. *Int. J. Mol. Sci. 20*(20).

Bhuiyan, Z. H., Chowdhury, M.K. (2016). Acitretin in dermatology a review. Bangladesh

Biewenga, G. P., Haenen, G. R., & Bast, A. (1997). The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol*, *29*(3), 315-331.

Braun, J., Rau R. (2009). An update on methotrexate. *Curr Opin Rheumatol*. *21*(3), 216-23.

Chan, E.S., Cronstein, B. N. (2010). Methotrexate--how does it really work? *Nat. Rev. Rheumatol*. *6*(3), 175-8.

Chande, N., Wang, Y., MacDonald, J. K., McDonald, J.W. (2014). Methotrexate for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* Aug 27;(8):CD006618.

Data, P. (1995). Alpha-lipoic acid. *Arzneimittelforschung*, *45*, 872-874.

Davis, S.A., Yentzer, B.A., Feldman, S. R., (2013). Acitretin prescribing patterns in women of childbearing potential. *J. Drugs Dermatol*, *12*(7):799-802.

Golbidi, S., Badran, M., Laher, I. (2011). Diabetes and alpha lipoic Acid. *Front Pharmacol*. *2*, 69.

Hannoodee, M., Mittal, M. (2022). Methotrexate. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan. PMID: 32310574 Bookshelf ID: NBK556114.

Holthoewer, D., Endres, K., Schuck, F., Hiemke, C., Schmitt, U., & Fahrenholz, F. (2012). Acitretin, an enhancer of alpha-secretase expression, crosses the blood-brain barrier and is not eliminated by P-glycoprotein. *Neurodegenerative Diseases*, *10*(1-4), 224-228.

Hung, S. Y., Boucias, D.G. (1996). Phenoloxidase activity in the hemolymph of naive and Beauvaria bassiana

infected Spodoptera exigua larvae. *J. Invert. Pathol. 67*, 35-40

Ighani, A., Partridge, A.C.R., Shear, N.H., Lynde, C., Gulliver, W.P., Sibbald, C., Fleming, P. (2019). Comparison of management guidelines for moderate-to-severe plaque psoriasis: A review of phototherapy, systemic therapies, and biologic agents. *J. Cutan. Med. Surg.*, *23*(2), 204-221.

Kahler, W., Kuklinski, B., Ruhlmann, C., Plotz, C. (1993). Diabetes mellitus-a free radical associated disease. Results of adjuvant antioxidant supplementation, *Z. Gesamte Inn. Med.*, *48*, 223- 232.

Khamaisi, M., Potashnik, R., Tirosh, A., Demshchak, E., Rudich, A., Tritschler, H., Bashan, N. (1997). Lipoic acid reduces glycemia and increases muscle GLUT4 content in streptozotocin diabetic rats. *Metabolism, 46*(7), 763-768.

Kilinc, G., Dursun, A., Tuncer, K., Esin, H., Emiroglu, M. (2021). Inflammatory Colitis due to methotrexate toxicity in a patient with psoriasis: a case report, *Med Records*, *3*(1),41-43.

Lagarce, L., Zenut, M., Lainé-Cessac, P. (2015). Methotrexate pharmacology. *J. Gynecol. Obstet. Biol.Reprod. (Paris), 44*(3),203-211.

Lotfi, M., Moniruzzaman, M., Mutalib, M. A., Wilfred, C. D., Alitheen, N. B., & Goto, M. (2016). Analysis of multiple solvation interactions of methotrexate and ammonium based ionic liquids using COSMO-RS. *Procedia Engineering*, *148*, 459-466. *Med. J* 45(2), 98-100.

Guenther, L.C., Kunynetz, R., Lynde, C.W., Sibbald, R.G., Toole, J., Vender, R., Zip, C. (2017). Acitretin use in dermatology. *J. Cutan. Med. Surg.*, *21*(3), 2-12.

Maritim, A.C., Sanders, R.A., Watkins III J.B. (2003). Effects of α-lipoic acid on biomarkers of oxidative stres in streptozotocin-induced diabetic rats. *J Nutr Biochem 14*(5), 288-294

Mehrtens, S.H., de la Hera, I., Shankar, S. (2018). Case of keratoacanthoma centrifugum marginatum treated with acitretin. *BMJ Case Rep*. Nov 01.

Molz, P., & Schröder, N. (2017). Potential therapeutic effects of lipoic acid on memory deficits related to aging and neurodegeneration. *Frontiers in pharmacology*, *8*, 849

Nomura, M., Sumiya, R., Ono, H. *et al.* (2021).Cessation of methotrexate and a small intestinal resection provide a good clinical course for a patient with a jejunum perforation induced by amethotrexate-associated lymphoproliferative disorder: a case report. *World J Surg Onc 19*, 4. https://doi.org/10.1186/s12957-020-02114-0

Ortiz, N. E. G., Nijhawan, R.I., Weinberg, J. M. (2013). Acitretin. *Dermatol Ther*, *26*(5), 390-9.

Packer, L., Cadenas, E. (2011). Lipoic acid: energy metabolism and redox regulation of transcription and cell signaling. *J Clin Biochem Nutr*. *48*(1), 26-32.

Reed L. J. (1998). From lipoic acid to multi-enzyme complexes. *Protein Sci. 7*, 220–224. 10.1002/pro.5560070125.

Rudrapal, M., Shubham, J., Kha, K. J., Dukhyil, A. B., Ansari, M.A., Alomar, M. N., Fahad M., Alshabrmi, F. M., Palai S., Prashanta Kumar Deb, P.K., Devi ajlakshmi Devi. (2022). Dietary polyphenols and their role in oxidative stress-ınduced human diseases: Insights Into protective effects, antioxidant potentials and mechanism(s) of action. *Front Pharmacol. 14*(13), 806470.

Shay, K. P., Moreau, R. F., Smith, E. J., Smith, A. R., Hagen, T. M. (2009). Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim. Biophys. Acta* 1790, 1149 1160. doi: 10.1016/j.bbagen.2009.07.026

Sadowska, M., Garbutt, J., Lesiak, A. (2022). Pros and cons of using systemic acitretin in the paediatric population. *Postepy Dermatol Alergol*. 39(1):34-38.

Schmidt, A.M., Hori, O., Brett, J., Yan, S.D., Wautier, J.L., Stern, D. (1994). Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler. Thromb*. 14, 1521–1528.

Sezgin, F.G., Dıraman, E. & İncilay Torunoğlu, E. (2022). Rat Beyin Dokusunda Asitretin –Metotreksat Kombinasyonunun ve A-Lipolik Asit'in Polifenol Oksidaz Aktivitesi Üzerine Etkileri. *European Journal of Science and Technology*, (37), 165-169.

Shiga, S., Machida, T., Yanada, T., Machida, M., Hirafuji, M., Iizuka, K. (2020). The role of nitric oxide in small intestine differs between a single and a consecutive administration of methotrexate to rats. *Journal of Pharmacological Sciences*. 143, 1, 30-38

Skillen, L.A, Corry, A. (2019). Combination therapy of sirolimus and acitretin in solid organ transplant recipients: a new cutaneous adverse event. *Clin Exp Dermatol*. 44(1):62-63.

Steffens, J.C, Harel, E, Hunt, M.D. (1994) Polyphenol oxidase. In BE Ellis, ed, Genetic Engineering of Plant Secondary Metabolism. *Plenum Press*, New York, pp 275–312.

Vallianou, N., Evangelopoulos, A., Koutalas, P. (2009). Alpha-lipoic Acid and diabetic neuropathy.*Rev Diabet Stud.* Winter 6(4), 230-6.

Wang, Y.H., Tsai, D. (2014). Small intestine perforation in a 58-year-old man with Darier disease after 25 months of oral acitretin therapy. *Dermatologica Sinica*. 32, 39-42

Weinblatt, M.E, Coblyn, J.S., Fox, D.A., Fraser, P.A., Holdsworth, D.E., Glass, D.N., TrenthamD.E. (1985). Efficacy of low-dose methotrexate in rheumatoid arthritis. *N. Engl. J. Med.*28;312(13):818-22

Zito, P.M, Mazzoni, T. (2022). Acitretin. In: StatPearls [Internet]. Treasure Island (FL): *Stat Pearls Publishing;* Sep 25.