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RESEARCH ARTICLE

Potential Therapeutic Effect of Lipoic Acid on Methotrexate-Induced Oxidative Stress in Rat Heart

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ABSTRACT

Objective: Methotrexate (MTX), an antifolate and antimetabolite, is used in the treatment of cancer and autoimmune diseases, however, it can cause many adverse events. Lipoic acid (LA) has anticancer and antioxidant activities, and due to these properties, it is effective in curing the complications of various disorders. The study aims to investigate the potential therapeutic effect of LA on MTX-induced oxidative stress in the heart tissues of rats.

Materials and Methods: Eighteen Wistar Albino rats were divided equally into 3 groups as follows: Controls, MT group (MTX was injected with a single dose of 20 mg/kg, i.p.) and MT+LA group (MTX was injected with a single dose of 20 mg/kg, i.p. on the first day and LA (dissolved in saline, 50 mg/kg/day, i.p,) was injected for 5 days). On the sixth day, rats were sacrificed under general anesthesia. Total protein, lipid peroxidation (LPO), nitric oxide (NO), sialic acid (SA), and glutathione (GSH) levels, and also catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase (GST) activities were determined in 10% (w/v) heart homogenates.

Results: MTX administration significantly increased LPO and NO levels, and SOD activity and significantly decreased GSH level and CAT activity. LA reversed these parameters by decreasing LPO and NO levels and SOD activity, and increasing GSH levels significantly.

Conclusion: LA has beneficial effects on the impaired oxidant/antioxidant status and is effective in reducing oxidative stress during MTX administration in the heart tissue of rats.

Keywords: Methotrexate, lipoic acid, oxidative stress, heart, rat

INTRODUCTION

Methotrexate (MTX), an antifolate and antimetabolite drug, is used in managing and treating cancer and autoimmune diseases.[1] It was originally designed as an antagonist of the folate pathway in leukemia treatment, and as a folate reductase inhibitor, it has anti-inflammatory, antiproliferative, antipsoriatic, and immunosuppressive effects. In addition to its immunosuppressive effect, MTX fights against neoplastic cells by blocking cell metabolism diseases.[2] As with many chemotherapeutic agents, MTX does not show high selectivity for damaged cells, so it is known to affect healthy cells as well. MTX can cause various toxicological effects and biochemical dysfunctions. In addition, various side effects of MTX, such as teratogenicity, infertility, hepatotoxicity, nephrotoxicity, and neurotoxicity have been reported.[3] Although the exact mechanism of MTX-induced toxicity is unknown, it is thought that it is a result of the formation of reactive oxygen metabolites (ROM) and a decreased antioxidant defense system due to MTX use.[4] Excessive production of ROM and other peroxide radicals increases oxidative stress in the cell and an imbalance between prooxidant and antioxidant defense occurs. Inadequate antioxidant defense mechanisms cannot protect the cell from oxidative damage resulting in cell damage.[5]

One of the alternative treatment methods used to reduce tissue damage during the treatment by chemotherapeutics is the use of antioxidant substances. Dietary antioxidants can enhance the deleterious effects of therapy by reducing or preventing some of the side effects of therapeutic drugs. For this purpose, many antioxidants have been tried and stated to be effective in reducing oxidative stress.[6,7] Lipoic acid (LA) is an endogenous substance found naturally in many common animal and plant foods. It readily crosses the blood-brain barrier and is accepted

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as a substrate by human cells. LA has anti-cancer and antioxidant activities, and due to these properties, it is effective in curing the complications of various disorders and has been used as a dietary supplement ingredient for many years.[8,9]

The study aims to investigate the potential therapeutic effect of LA on MTX-induced oxidative stress in the heart tissues of rats.

MATERIALS AND METHODS

The experimental procedures were approved by the Marmara University Animal Care and Use Committee (Protocol Number: 21.2023mar). Eighteen male Wistar Albino rats (200-250gr, 2 months old) were housed under standard laboratory conditions and given water ad libitum and standard rat pellets throughout the study. MTX (50 mg/5 mL) was obtained from Koçak Farma İlaç ve Kimya Sanayi A.S., Turkey, and LA (catalog number: 1077-28-7) was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Study Group

Rats of the experiment were randomly divided into 3 groups as follows: Control group (C) (n=6), MTX administered group (MT) (n=6), and MTX+LA administered group (MT+LA) (n=6). In the MT group, a single dose of MTX was injected (20 mg/kg, i.p.) on the 1st day and saline was injected (0.1 mL/100 g/day, i.p.) for 5 days. In the MT+LA group, a single dose of MTX (20 mg/kg, i.p.) was injected on the 1st day, and LA (dissolved in saline, 50 mg/kg/day, i.p.) was injected for 5 days. On the 6th day of the experiment, all rats were sacrificed under general anesthesia with sodium pentothal (50mg/kg, i.p.), and heart tissues were taken immediately for biochemical examination. Homogenates (10% w/v) were made and kept at -20°C.

Biochemical Parameters

Biochemical parameters; total protein, lipid peroxidation (LPO), nitric oxide (NO), sialic acid (SA), and glutathione (GSH) levels, additionally catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase (GST) activities were done in heart homogenates of rats.

Total protein levels of the heart tissues were measured by the method of Lowry et al.[10] and used to express the results of the parameters per protein. LPO level was measured by the method of Yagi[11] and the results were presented as nmol MDA/mg protein. The method of Miranda et al.[12] was used to detect NO levels and the results were expressed as nmol/mg protein. SA levels were determined by the method of Warren[13] and the results were presented as mg/g protein. The method of Beutler[14] was used to determine GSH levels and results were expressed in mg/g protein. CAT activity of the heart tissues was evaluated by the method of Aebi[15] and the results were presented as U/mg protein. SOD activities of the heart tissues were determined by the method which is based on its ability to increase the effect of riboflavin-sensitized photooxidation of o-dianisidine[16] and the results were expressed in U/g protein. GST activity was evaluated by the method of Habig[17] and the results were expressed as U/g protein.

Statistical Analysis

All data was analyzed by using the program GraphPad Prism 9 (GraphPad Software, San Diego, USA). Shapiro-Wilk test was used to confirm the normal distribution of the data, and the one-way analysis of variance (ANOVA) test and Tukey's multiple comparison tests were applied. Data is presented as mean±standard deviation and a value of $p<0.05$ indicates that there is a significant difference.

RESULTS

Oxidation Parameters

Heart LPO, NO and SA levels of the groups are shown in Figure 1. LPO and NO levels of the MT group significantly increased compared with the controls, and significantly decreased compared with the MT+LA group ($p<0.01$, $p<0.05$, $p<0.05$, $p<0.05$, respectively). Besides, heart SA level in the MT group increased compared with the C group, and decreased compared with the MT+LA group, but the results were insignificant.

Antioxidant Parameters

Heart GSH level and CAT, SOD, and GST activities of the groups are shown in Figure 2. In the MT group, a significant decrease in GSH level and CAT activity were found compared with the controls ($p<0.01$, $p<0.05$), and GSH level significantly increased compared with the MT+LA group ($p<0.05$). Besides, a slight increase was noticed in the CAT activity of the MT+LA group compared with the MT group, but the result was insignificant. Heart SOD activity significantly increased in the MT group compared with the C group, and significantly decreased compared with the MT+LA group ($p<0.01$, $p<0.05$). Neither MTX administration nor MTX+LA administration changed GST activity in the heart tissue of the rats.

DISCUSSION

MTX is one of the most used antifolate and antimetabolite drug.[1,18,19] However, many adverse events occur due to the use of MTX during treatment. These side effects are generally dose-dependent or related to the usage period.[20] Although the mechanism has not been fully clarified yet, previous studies have put forward excessive ROM production during MTX therapy, and this condition is one of the major causes of MTX-induced side effects.[21,22] MTX inhibits the NAD^+-dependent

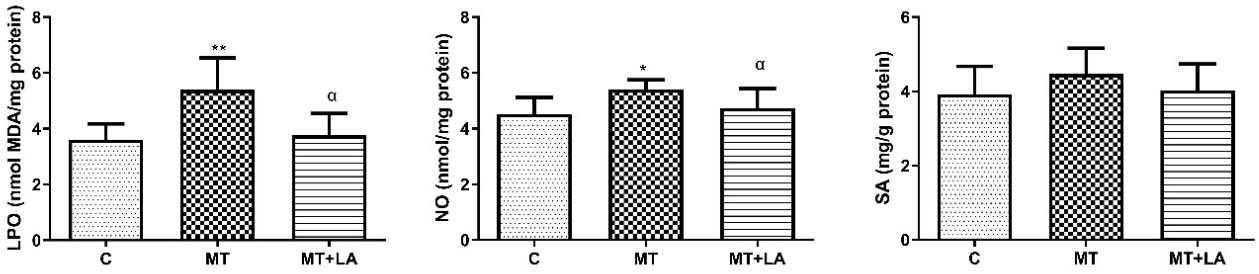

Figure 1. Levels of LPO, NO, and SA in heart tissue. Values are given as mean±standard deviation, C: Control group; MT: Methotrexate given group; MT+LA: Methotrexate and Lipoic acid given group; LPO: lipid peroxidation; MDA: malondialdehite; NO: nitric oxide; SA: sialic acid. *p<0.05, **p<0.01 significantly different from group C; ^{a}p<0.05 significantly different from group MT.

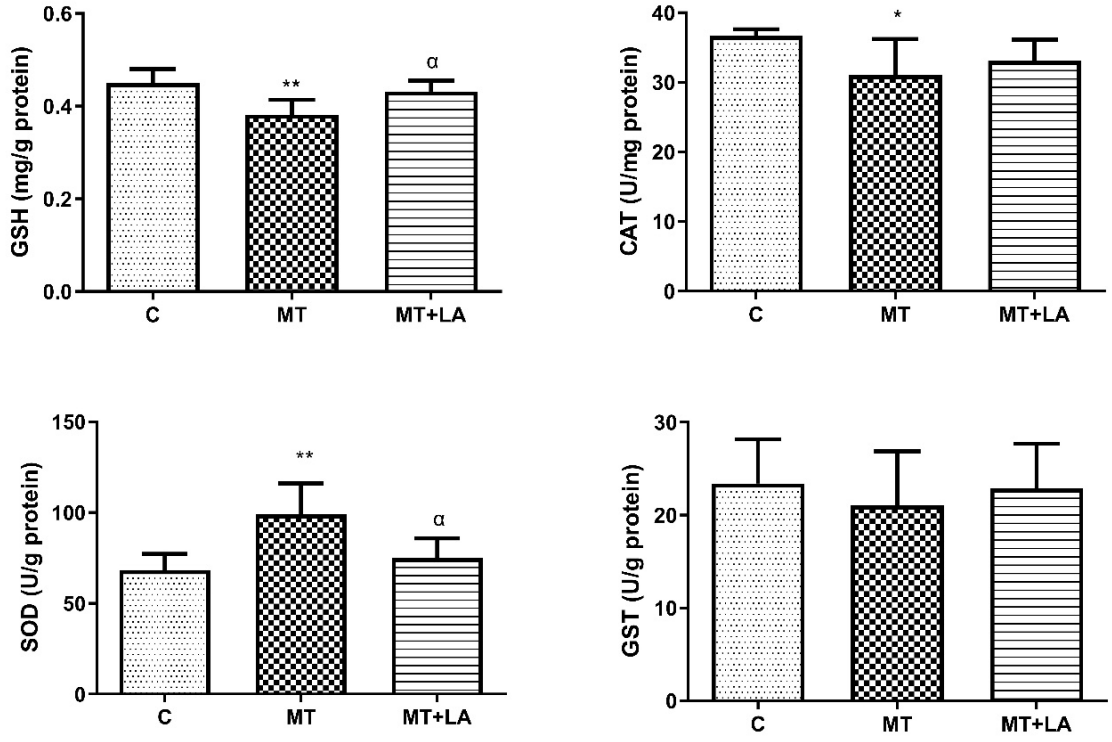

Figure 2. Level of GSH, activities of CAT, SOD, and GST in heart tissue. Values are given as mean±standard deviation, C: Control group; MT: Methotrexate given group; MT+LA: Methotrexate and Lipoic acid given group; GSH: glutathione; CAT: catalase; SOD: superoxide dismutase; GST: glutathione-S-transferase. *p<0.05, **p<0.01 significantly different from group C; ^{a}p<0.05 significantly different from group MT.

mitochondrial enzymes, impairs the defense system, and thus it causes the cells to be unable to resist free radicals. Besides, MTX depletes protective antioxidant defense molecules and inhibits the activities of free radical scavengers.[23]

Excessive ROM production and increased LPO levels due to radical formation induce cellular injury by damaging the cell membrane. Based on the previous knowledge, 20 mg/kg of MTX was used in the present study to induce MTX-related tissue damage, and LPO level significantly increased in the MT group, by previous data.[24,25] The increase in ROM may have overpowered the antioxidant system and damaged the cell, which could have been detected as an increase in the LPO level. NO is a molecular mediator of various pathological and physiological processes, such as vasodilation and inflammation. Overproduction of NO in the presence of oxidative stress contributes to tissue damage by interacting with superoxide and forming

peroxynitrite, a potent cytotoxic agent.[25] As in elevated LPO level, NO level also increased significantly in the MTX-given group than those of the controls. Other studies also support our findings.[26,27] The increase both in LPO and NO levels of the MT group shows that MTX triggers oxidative stress, exposing cells to radicals and making them prone to cellular damage. SA is one of the terminal sugars in many glycoconjugates and is present in all biological membranes. It has a crucial role in cell protection by increasing in pathological situations. Also, SA is a stable marker of inflammation with less intra-individual variability, and therefore, it can more accurately represent inflammatory status.[28] In the present study, MTX injection elevated SA levels but the result was insignificant, which may be caused by the heart tissue's self-protection against radical damage mediated by MTX administration. The explanation of oxidative stress-induced damage in heart cells may be a reason for the imbalance between antioxidants and oxidants. There may be a loss or insufficiency of antioxidant defense. Depletion of antioxidant enzymes and defense mechanisms in the cell causes mitochondrial dysfunction and induction of endoplasmic reticulum stress due to increased ROM production.

GSH is an important antioxidant due to its involvement in cellular metabolism and reducing properties. In previous studies, MTX-mediated oxidative stress was pointed out as a factor for the depletion of GSH in rats.[25,29] MTX inhibits the activity of malic enzymes and causes a decrease in NADPH. As a result, the activity of GR decreases, which is involved in GSH metabolism and uses NADPH as a cofactor, thus reducing the conversion of GSSG to GSH.[30] In parallel with this information, significantly decreased GSH levels were observed in MTX-injected group, which means MTX may cause a depletion in GSH reservoirs. SOD is an intracellular antioxidant enzyme. It reduces superoxide anion to hydrogen peroxide and protects tissues against reactive metabolites. It is a major defense system against the toxic effects of oxygen radicals.[31] In the present study, MTX injection elevated SOD activity in heart tissues. The result may be a sign that the cell continues to protect itself, since a single dose of MTX injection was not sufficient to deplete the SOD enzyme and the cell may tolerate the dose. CAT and GST enzymes also have antioxidant properties in detoxifying hydrogen peroxide to water. According to previous studies, MTX-induced toxicity decreases the level of antioxidant enzymes in the tissues.[32] MTX injection to the rats caused a significant decrease in CAT activity and a slight decrease in GST activity in heart tissue which may be the result of the depletion of these enzymes or loss of their activity by MTX injection.

There have been several studies regarding the use of antioxidant supplementation to reduce MTX-induced toxicity and its effect on MTX efficacy in diseases.[33] LA is an effective molecule that has a high antioxidant capacity. Both oxidized and reduced forms of LA have antioxidant effects by eliminating and/or reducing reactive metabolites. The disulfide bond of LA reacts with radicals, eliminates radicals that trigger LPO, and then inhibits the initiation and progression of LPO induced by hydrogen peroxide radicals.[34] It has been shown in many studies that LA fights oxidative stress by scavenging free radicals.[8,9,34] In addition to its role in scavenging and eliminating the metabolites of ROM, LA also aids in the formation of other cellular antioxidants, such as vitamins C and E.[35] Given these features of LA, it is useful in reducing cellular MDA and NO dose-dependently.

In the present study, LA administration reduced LPO and NO levels significantly in the MT group, and slightly decreased SA level. Besides, LA increased the level of GSH, which decreased with MTX administration. LA supports the insufficient level of GSH as a result of excessive oxidant formation by reducing cellular damage. Also, the LA administration decreased SOD activity in the MT group. With the reduction of ROM production, the superoxide anion may also have decreased in the medium and the SOD activity may have reversed to the control level. Although LA did not change CAT and GST activities significantly, a slight increase was observed in these parameters, which may be the effect of LA on supporting the antioxidant defense system and reversing the damage in the cell by changing the antioxidant levels.

CONCLUSION

LA has beneficial effects on the impaired oxidant/antioxidant status and is effective in reducing oxidative stress during MTX administration in the heart tissue of rats.

Ethics Committee Approval: The experimental procedures were approved by the Marmara University Animal Care and Use Committee (Protocol Number: 21.2023mar).

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