

Effects of alpha lipoic acid on level of NO and MPO activity in diabetic rats

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Abstract

Introduction: Alpha lipoic acid (ALA) is a natural antioxidant including anti-diabetic properties and it is effective in improving the complications of diabetic nephropathy.

Objectives: In this study, the beneficial effects of ALA on serum and liver myeloperoxidase activity and serum level of nitric oxide in alloxan-induced diabetic rats were investigated.

Materials and Methods: In this study randomly 30 male Sprague Dawley rats were divided into 3 groups; first controlling groups, second untreated diabetic, third diabetic rats treated with daily injections 100 mg/kg ALA. In second and third groups, diabetes was induced by injections of 100 mg/kg subcutaneously alloxan. Having treated in 6 weeks, the animals were anesthetized, heart phlebotomizing was done in order to measure level of NO and myeloperoxidase (MPO) activity in serum. Then liver was excised quickly and kept in the freezer -70°C so as to measure myeloperoxidase activity.

Results: Compared to the untreated diabetic group, ALA significantly reduces myeloperoxidase activity on serum and liver, decreased serum levels of NO in the treatment group.

Conclusion: The results show that ALA may have anti-inflammatory properties and it may improve liver function in diabetic damages induced by oxidative stress. ALA also may have beneficial effects in reducing complications of diabetics as a strong antioxidant.

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Introduction

Oxidative stress caused by hyperglucosemia is effective in the pathophysiology of different diabetic complications (1). Various studies show that oxidative stress and free radicals have an essential role in diabetic complications such as diabetic nephropathy (2). In diabetes mellitus, oxygen free radicals are produced through auto-oxidation of glucose. Oxidative damage results from an imbalance between formed and disabled free radicals. Disabled and removed radicals are associated with antioxidant defense mechanisms (3). In granular azarophyllic, enzyme myeloperoxidase (MPO) involved in the germicidal process is containing iron. In order to response to vascular injury such as atherosclerosis, myeloperoxidase has a significant function. Accordingly it is known as a biological marker in inflammatory diseases (4).

Nitric oxide (NO) is a signaling molecule with cellular effects, cardiovascular and metabolic process involved in many biological

Core tip

Our study shows ALA reduced the level of nitric oxide and myeloperoxidase activity in diabetic treated group. The authors hope, the results of the present study help to improve inflammation and the complication in diabetic patients.

regulation such as cell proliferation, neurotransmitter, antimicrobial defense, vasodilatations, inflammatory response (5,6). Therefore MPO and NO affect the process of inflammation by molecular mechanisms and reaction between them is a sign of local inflammation and cardiovascular events initiation and progression in body (6). Many drugs and antioxidants have anti-inflammatory effects that among them natural antioxidants are more useful due to their adverse effects is low (7).

Natural antioxidant has anti-inflammatory, hypoglycemia and hyperlipidemia activity and they conserve cells against oxidative stress by transforming toxic free radicals

into nontoxic products. Therefore using antioxidants will be effective in treating illnesses associated with oxidative stress (8). Alpha lipoic acid (ALA) is disulfide compound involved in pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase reactions and as enzyme and it is effective in cellular energy production (3). ALA is an antioxidant with potential acute treatment in most diseases such as diabetes mellitus and its complication, high blood pressure, Alzheimer, Dawoon syndrome, cognitive impairment, kinds of cancer (9).

ALA is effective in reducing oxidative stress by preventing lipid peroxidation and protein damage as other antioxidants significantly inhibit the reactive oxygen species such as proxy nitrite, nitric oxide, hydroxyl radicals, superoxide anion in the membrane and aquatic environment (10,11). ALA is an antioxidant androgen causes reconstruction by reducing the oxidized form of them such as vitamin E, C and glutathione, thus it has a function in boosting antioxidant effect (12). ALA has inflammatory properties, protective effect on the development of atherosclerosis and inhibits the progression of created atherosclerotic plaques (13). Given that very few studies have been done in rats with type 1 diabetes about ALA protective effects on inflammation and oxidative damage caused by diabetes. The purpose of this study is investigation of protective effects of ALA on MPO and NO activity in alloxan-induced diabetic rats.

Objectives

According to the fundamental role of ROS in pathogenesis of diabetes, ALA an antioxidant agent for the first time was used in combat diabetes. No detailed research has been carried out on the efficiency of ALA in the modulation of oxidative stress associated with diabetes complications.

Material and Methods

Laboratory animals

Current study has been done on 30 male Sprague-Dawley rats with an average weight of (180-200 g) purchased from Tehran Pasteur Institute. Animals were kept in the lab animals under controlled conditions at $23 \pm 2^\circ\text{C}$ and 12 hours of light, 12 hours of darkness in lighting conditions so as to adjust to the new environment. The animals were given enough food and water in entire of the study. The entire process of working with animals was done based on using of laboratory animals ethics committee of Lorestan University of medical sciences which is in accordance with the laboratory instructions for caring and using of laboratory animals. The rats were randomly divided into three groups: first, controlling rats, second untreated diabetic rats and third diabetic rats treated with ALA.

Diabetes induction

Diabetes induction Second and third groups of rats were diabetic by injection of 100 mg/kg subcutaneously alloxan monohydrate after a day of fasting sucrose solution 10% was replaced by drinking water for 48 hours so as to create acute hypoglycemia (14). Five days after induction

of diabetes, blood glucose was measured by a glucometer. Rats having a glucose level of $> 300 \text{ mg/kg}$ (16.7 mmol/L) were separated as diabetes (2). Third group was daily treated by 100 mg/kg ALA injection for 6 weeks. Having made unconscious the animals by ketamine (75 mg/kg i.p.), after the end of treatment, phlebotomizing was done. Blood samples were kept at room temperature for 20 minutes so as to clot. Then 3000 rpm centrifuge for 20 minutes in a round serum was isolated from blood samples (2,15). Also animal's liver was isolated immediately and was kept in freezer at -70°C until testing.

Biochemical studies

Measuring level of NO

Serum levels of nitrite were measured by Griess reaction with sodium nitrate as the standard. Around $50 \mu\text{L}$ of serum sample was mixed in microplate with 100 μL reagent grace (containing sulfanilamide 1%, *N*-1-aminoethyl naphthylamine dihydrochloride 1% in phosphoric acid 5%). It was kept at temperature laboratory, and then absorbance at 540 nm was read. Nitrate thickness was calculated by comparing with sodium nitrate standard diagram ($\text{NaNO}_2 = 0-110 \mu\text{mol/L}$) (16).

Measuring activity of MPO

Myeloperoxidase activity was measured by little changing based on Bradly method. 0.3 mL potassium phosphate buffer 0.1 M ($\text{pH} = 6$), 0.3 mL hydrogen peroxide 0.01 M, 0.5 mL O-dianisidine 0.02 M and 10 μL (serum or homogenized liver) in a way that the ultimate volume reaches 3 mL were mixed in a pile 1 cm in diameter. Absorbance mixed changes in pile was noted down in 460 nm wavelength for 10 minutes. Eventually MPO activity level was reported according to unit/mg protein (17).

Ethical issues

Prior to the experiment the Animal Ethics Committee of the Medical University of Lorestan approved the protocol and confirmed that it is in accordance with the national health and medical research council guidelines. The principles of Animal Ethics Committee of the Medical University of Lorestan founded on the tenets of the Declaration of Helsinki.

Statistical analysis

All data were represented in a ($\text{mean} \pm \text{SEM}$) manner for testing groups. They were compared so as to investigate their differences by one-way analysis of variance (ANOVA) with Duncan U test. Difference between statistic groups is meaningful in case of P value < 0.05 . All statistical analysis is doing by SPSS version 22 Windows software.

Results

ALA effects on NO serum in diabetic rats

Serum levels of NO are shown in Figure 1. Serum NO level in untreated rats significantly has increased compared to controlling group (1.21 fold). Moreover, treating trough

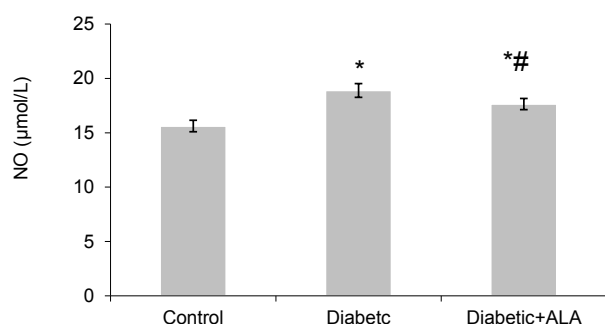


Figure 1. The effect of alpha lipoic acid on serum level nitric oxide in alloxan induced diabetic rats.

* $P < 0.05$ as compared with control group.

$P < 0.05$ as compared with diabetic without treatment group.

ALA significantly decreases serum NO level (7.10%) in treated group compared to diabetic group.

ALA effects on serum and liver of MPO in diabetic rats

The activity of MPO in serum is shown in Table 1. Serum MPO activity significantly has increased in diabetic group compared to healthing group (4.52-fold). Treating trough ALA decreases (55.79%) MPO serum activity in treated diabetic group compared to untreated diabetic group. The activity of MPO in liver is shown in Table 1. Liver MPO activity significantly has increased in diabetic group compared to healthing group (2.11-fold). Treating trough ALA decreases (50.52%) MPO liver activity in treated diabetic group compared to untreated diabetic group.

Discussion

This study shown that in diabetic rats, ALA has useful effects on decreasing inflammatory and biochemical markers. Findings suggested that ALA controls increased NO and MPO activity in diabetic groups. Varies studies suggest that diabetic causes producing active kinds of oxygen and extra glucoses enters the polyol and glucosamine pathway and lead to form and active protein kinase AGE, C and glycation. Consequently, extracellular proteins synthesis and increased expression of inflammatory mediators lead to tissue damage (1). Therefore stress oxidative functions as a common communicative factor in the majority of pathogenic pathway caused by diabetes (18). Laboratory findings have suggested that treating through antioxidants and controlling diabetes in rodents (19). Antioxidant such

as E and C vitamins (20), carvacrol (21) and coenzyme Q10 (2) have preservation effects on neutralizing of free radicals and decreasing oxidative damages.

The effect ALA on NO and MPO activity

Finding of current study suggested that serum NO activity and liver and serum MPO activity in diabetic rats have increased compared to controlling rats. Treating trough ALA considerably decreases serum NO and liver and serum MPO activity compared to untreated rats. NO is lipophilic small molecules has a significant function in majority of biological processes such as inflammation, cell proliferation, neurotransmitter and oxidative stress (5). Metabolism NO adjustment in diabetes type 2 is significant since NO synthase activation is under insulin control through PI3K-AKT (22). It has been showed that varied antioxidant such as α -tocopherol, quercetin (20), melatonin, and apocynin (23) have also effects on serum NO level as ALA. Also there are reports representing E vitamin, selenium and L-arginine increase NO serum (20). MPO is a biomarker of inflammatory diseases. It has a significant function in responding to vascular damage in inflammatory diseases (CVD) and in beginning atherosclerosis improvement and side effects (24). Similar studies suggested that MPO enzyme level in diabetes increases which has a significant function in atherosclerosis improvement (25). There are reports representing oleuropein strongly controls MPO enzyme level in inflamed tissues (19). Also other studies findings represent ALA function in improving HbA_{1c}, lipid peroxidation, antioxidant enzymes and inflammatory markers (26). ALA has a function in decreasing damages caused by diabetic neuropathy and inflammatory markers as a compound with strong antioxidant potential (27). Findings of this study which suggested antioxidant effects of lipoic acid in increasing NO level and MPO activity are similar to findings of antioxidant ALA effects reported by other researchers. Therefore treating diabetes based on hyperglucosemia and oxidative stress increase may consider as a secure and affordable solution in prevention and treatment of diabetes which this treatment approach can be possible by utilizing antioxidants and herbal extracts containing antioxidant effects.

Conclusion

This study suggested that ALA has useful protective effects in reduction of NO serum and MPO activity level as inflammatory on liver and serum, in alloxan- induced diabetic rats. Therefore it may be useful to control NO and MPO level in patients suffer with vascular diseases, atherosclerosis and diabetic damages.

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Authors' contribution

All authors wrote the manuscript equally.

Table 1. The effect of alpha lipoic acid on serum and liver myeloperoxidase activity in alloxan induced diabetic rats.

Groups	Serum MPO (U/mg protein)	Liver MPO (U/mg protein)
Control	88.72 ± 44.5	135.4 ± 19.47
Diabetic	401.7 ± 128.1*	357.7 ± 46.7*
Diabetic treated	177.6 ± 36.8*#	175.06 ± 13.2*#

Values represented as mean ± SD.

* $P < 0.05$ as compared with the control group.

$P < 0.05$ as compared with the diabetic without treatment group.

Conflicts of interest

The authors declared no competing interests.

Ethical consideration

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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