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Research Article

Protective effect of multiple administration of alpha – lipoic acid in lipopolysaccharide – induced model of inflammation in rats

Nina Doncheva[1], Lilia Vasileva[1,3], Maria Ivanovska[2], Atanaska Petrova[2], Mariana Murdjeva[2], Damianka Getova[3]
[1]Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University-Plovdiv, Vassil Aprilov Str. 15A, Plovdiv 4002, Bulgaria
[2]Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University-Plovdiv, Vassil Aprilov Str. 15A, Plovdiv 4002, Bulgaria
[3]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University-Plovdiv, Vassil Aprilov Str. 15A, Plovdiv 4002, Bulgaria

***Corresponding author**
Nina Doncheva
Email: ninanina1972@abv.bg

Abstract: Alpha-lipoic acid is well known antioxidant and has anti-inflammatory effects. It is used to treat diabetic neurovascular and metabolic problems. The aim of our study was to evaluate the anti-inflammatory effect of alpha- lipoic acid (ALA) in lipopolysaccharide (LPS) - induced model of inflammation in rats. Forty male Wistar rats were divided in five groups (n=8): control, model group and three experimental groups treated with 30, 60 and 90 mg/kg alpha – lipoic acid for 14 days. The inflammation was induced by a single dose administration of LPS from Escherichia coli 055: B in dose 250 µg/kg i.p. After four hours the animals were sacrificed and blood samples were collected. The levels of two pro-inflammatory factors including tumor necrosis factor- alpha (TNF-alpha) and interleukin -6 (IL-6) were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA). Intraperitoneal administration of LPS increased the level of both TNF-alpha and IL-6 in the model group compared to the control group. In all ALA treated groups the level of TNF-alpha was significantly decreased ($p<0, 05$) compared to the model group. In contrast, IL-6 release in the group treated with 30 mg/kg ALA was significantly ($p<0, 05$) higher than the model group. There was no significant reduction ($p<0, 05$) in the level of IL-6 in all experimental groups. Our present study demonstrates that chronic ALA administration significantly protects against lipopolysaccharide-induced inflammation in rats.
Keywords: alpha- lipoic acid, lipopolysaccharide, inflammation, TNF-alpha, IL-6, rats.

INTRODUCTION

Inflammation is a defense mechanism in which leucocytes migrate from the vasculature into damaged tissues to destroy the agents that potentially can cause tissue injury. Acute inflammation is a limited beneficial response whereas chronic inflammation is a persistent phenomenon that can lead to tissue damage. While in acute inflammation the leucocyte infiltrate is composed mostly of neutrophils, chronic inflammation is histologically associated with the presence of mononuclear cells, such as macrophages and lymphocytes [1].

Alpha - lipoic acid (ALA) as a multifunctional antioxidant is effective at ameliorating symptoms in diseases with an underlying oxidative stress component [2]. Therapeutic potential of ALA has been reported in a variety of diseases, including diabetes mellitus, cardiovascular diseases and cancers [3, 4]. Alpha - lipoic acid is an antioxidant able to produce its effects in aqueous or lipophilic environments. It presents a highly negative reduction potential, increases the expression of antioxidant enzymes and participates in the recycling of vitamins C and E. Due to these properties, ALA is called the "universal antioxidant". ALA is also involved with anti-inflammatory effect, independently of its antioxidant activity. Alpha - lipoic acid significantly improves diabetic neurovascular and metabolic abnormalities and may play role as an anti-inflammatory agent [5].

Endotoxin, or lipopolysaccharide (LPS), is a component of the cell wall of Gram negative bacteria and is released into the host environment by the destruction of the cell wall. Endotoxin binds to LPS-binding protein which leads to initiation of inflammatory response, activation of macrophages and monocytes, and production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and IL-12, IL-1β, [6], free radicals and reactive oxygen species [7]. These cytokines can activate the NF-κβ pathway, which plays

a central role in inflammation through the regulation of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS). This pathway is activated upon appropriate extracellular stimulation, most often by stress or pro-inflammatory cytokines, including TNF-alpha and IL-1, and by pathogens such as bacterial components including lipopolysaccharide (LPS) through the Toll-like receptors (TLRs) [8].

TNF-alpha plays key role in body immune defense by providing protection against many pathogens. It is a cytokine generated by a variety of different cells- most effectively y mononuclear phagocytes- in response to a wide range of immune stimuli and stress conditions [9]. TNF-alpha as one of the major regulators of inflammation causes fever, increases vascular permeability and stimulates liver production of acute-phase proteins [10].

Among the cytokines of first discovery is IL-6, which is still a subject of intensive investigations today because of its ubiquity and functional diversity [11]. Indeed, IL-6 was found to be produced by cells at tissue sites and released into circulation in such acute situations of homeostatic perturbation as endotoxemia, endotoxic shock or trauma. In these processes, circulating IL-6 has been known to play an important role in the induction of acute phase reactions [12]. IL-6 production is induced together with other alarm cytokines TNF-alpha and IL-1 that are also involved in the elicitation of acute phase reactions. It has remained unclear whether IL-6 is merely an acute phase reaction-inducing cytokine or has any additional pro-inflammatory and anti-inflammatory activities in these processes. Indeed, the lack of understanding of other functional aspects of this cytokine in both local and systemic acute inflammatory responses has led to a confounding description about the nature of endogenous IL-6 in literature which has been described as either pro-inflammatory or anti-inflammatory.

MATERIAL AND METHODS

Animals

Experiments were performed on 40 male Wistar rats weighing 200–220 g. The animals were housed 8 per cage under standard laboratory conditions of 12/12 h light-dark cycle. All animals received a standard diet and water ad libitum.

The experimental procedures followed the guidelines for the care and use of laboratory animals, and they were approved by the Scientific Ethics Commission of the Medical University, Plovdiv, Proceedings №4/25.06.2015.

Drugs

Alpha – lipoic acid (Worwak Pharma, Germany)

LPS from Escherichia coli 055: B (Sigma)

Experimental design

Animals were randomly divided into five groups and treated as follows:

Group I (control group) – saline 0, 1 ml/ kg

Group II (model group) – saline 0, 1 ml/ kg + LPS 250 μg/kg

Group III– alpha-lipoic acid 30 mg/kg + LPS 250 μg/kg

Group IV – alpha-lipoic acid 60 mg/kg + LPS 250 μg/kg

Group V – alpha-lipoic acid 90 mg/kg + LPS 250 μg/kg

All agents were dissolved in saline as a vehicle and injected intraperitoneally (ip) for 14 days. LPS was injected on day 15 and 4 hours later blood samples were collected. The blood samples were centrifuged at 3000 rpm for 10 min to separate the serum from the cloth. Average volume from 1, 5 ml to 2, 5 ml serum was transferred in eppendorf tubes. To detect the anti-inflammatory effect of ALA we studied the levels of the cytokines TNF-alpha and IL-6 in blood serum.

LPS-induced inflammation model

LPS was dissolved in sterile normal saline and was injected intraperitoneally in dose 250 μg/kg four hours before blood collection.

TNF-alpha and IL-6 determination

The concentration of TNF-alpha and IL-6 in serum was measured by specific enzyme-linked immunosorbent assay (ELISA). The test was performed using the Rat IL-6 ELISA KIT, Diaclone and Rat TNF-alpha ELISA KIT, Diaclone strictly following the manufacturer's recommendations. The reaction was red at 450 nm and reference filter at 620 nm. Minimal detection levels were 19 pg /ml for IL-6 and 15 pg/ ml for TNF- alpha.

STATISTICAL ANALYSIS

SPSS 17.0 statistical software was used. All values are expressed as mean ± SEM. Differences between mean values were analyzed with independent sample t-test or Mann-Whitney test depending on the results from Kolmogorov-Smirnov normality test. Statistical significance was accepted at $P < 0, 05$.

RESULTS

I. TNF-alpha rat serum levels after multiple administration of alpha – lipoic acid

Intraperitoneal administration of LPS increased TNF-alpha level in the model group ($p<0.01$) compared to the control group. In LPS-induced model of inflammation ALA at all tested doses decreased significantly ($p< 0, 05$) the serum level of TNF-alpha compared with the model group (Figure. 1).

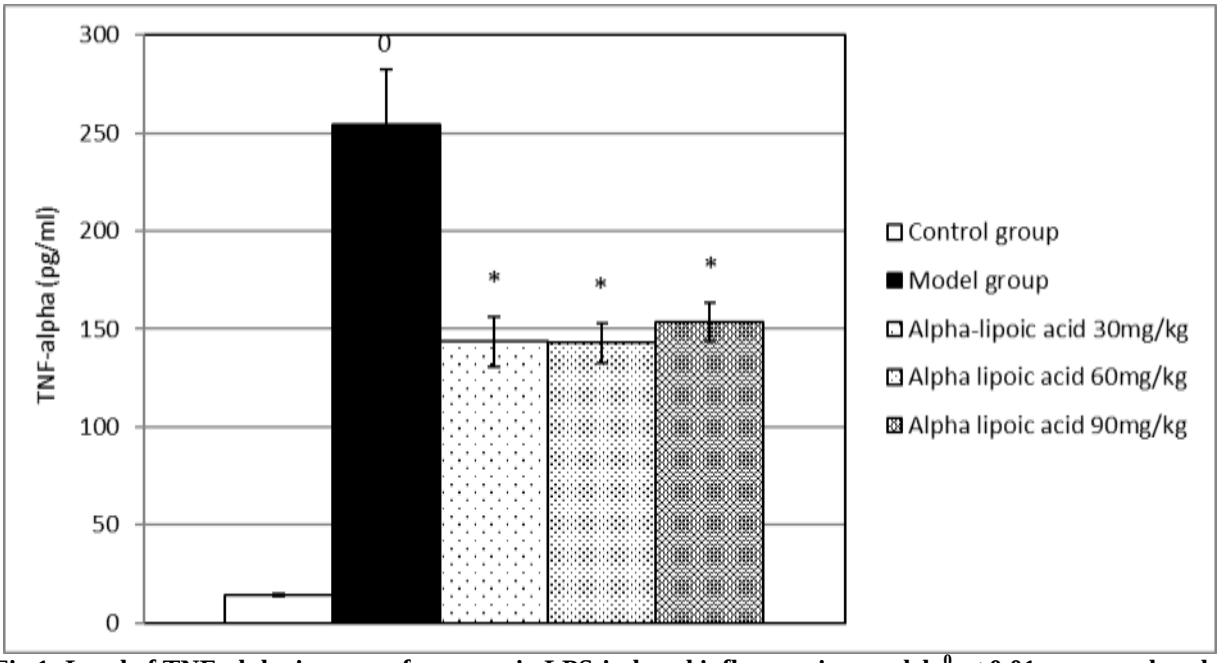

Fig 1: Level of TNF-alpha in serum from rats in LPS-induced inflammation model. $^{0}p< 0.01$ compared to the control group; $^{*}p < 0.05$ compared to the model group

II. IL-6 rat serum levels after multiple administration of alpha – lipoic acid

Injection of LPS caused a significant increase in the IL-6 level of the model group ($p< 0.05$) compared to the control group. In LPS-induced model of inflammation ALA at a dose 30 mg/kg increased significantly the level of IL-6 ($p<0, 05$) compared with the model group. ALA at higher doses (60 and 90 mg/kg) showed no change in the level of IL-6 compared to the model group (Figure. 2).

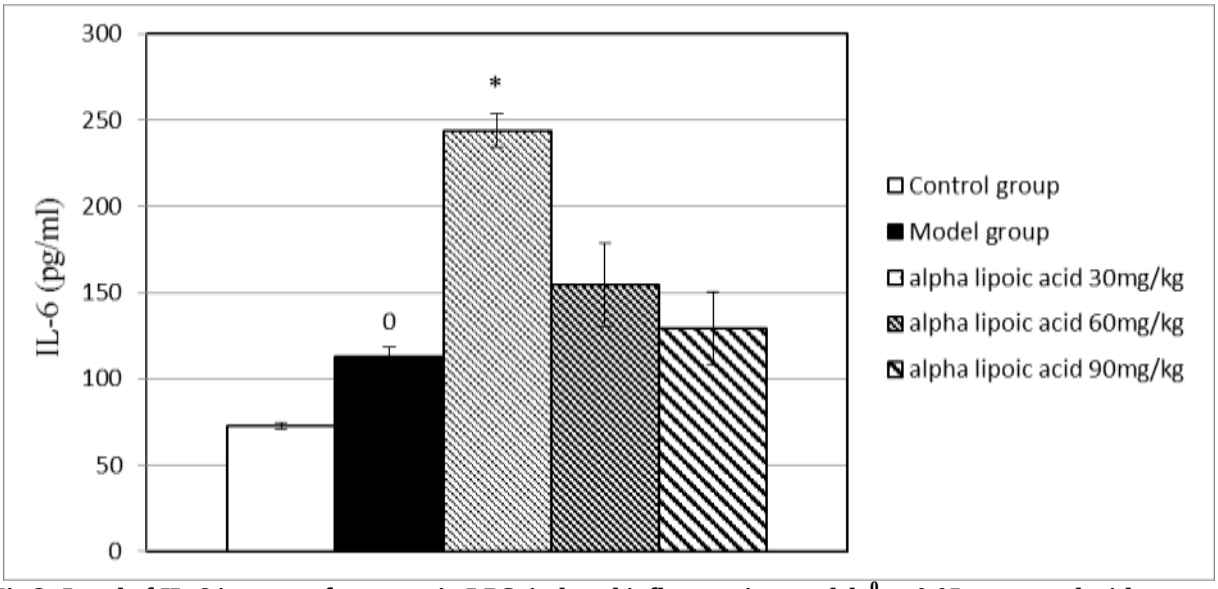

Fig 2: Level of IL-6 in serum from rats in LPS–induced inflammation model. $^{0}p< 0.05$ compared with control group; $^{*}p < 0.05$ compared with model group

DISCUSSION

In the present study the effect of ALA on LPS-induced inflammation model in rats was observed. LPS triggers the inflammatory response, leading to the release of large numbers of endogenous inflammatory mediators, including tumor necrosis factor-alpha, interleukins (IL-4, IL-10, IL-13, IL-1), chemokines, adhesion molecules, reactive oxygen species (ROS), and reactive nitrogen species [13]. As a component of Gram-negative bacterial cell wall, LPS is one of the most potent activators for regulating gene expression of inflammatory cytokines via the NF-kB pathway and inducing nitric oxide (NO) and reactive oxygen species (ROS) generation [14, 15].

Our data support that LPS administration in rats induces pronounced inflammatory response evidenced by an increase in the levels of TNF-alpha and IL-6 compared to untreated control which confirms the validity of the used model.

It is known that binding of cytokines as TNF-alpha to a specific receptor triggers oxygen shock in the cell, resulting in a rise in cellular level of ROS, which transmit the signal to transcription factor, as NF-κB, that activates expression of specific genes. NF-κB is a redox-sensitive transcription factor, which plays a role in the expression of a variety of genes that are involved in inflammatory response and in apoptosis in multiple tissues and cell types. According to Chibu S [16] TNF-alpha is a key activator of the NF-κB pathway, which mediates inflammatory responses and regulates the expression of several inflammatory mediators, including chemokines, cytokines and cytokine receptors.

In our results ALA at all doses used decreased the serum levels of TNF-alpha after multiple administrations. This data is in agreement with the reports of the anti-inflammatory action of ALA in various inflammatory diseases [17, 18, 19, 20]. These effects might be related to the inhibition of NF-κB activation through decreased oxidative stress and subsequent reduction in pro-inflammatory chemical mediators [19]. Holmquist *et al.;* [21] have demonstrated that ALA suppresses the NF-κB-dependent up-regulation of intracellular adhesion molecules (ICAM), TNF-alpha and monocyte chemo attractant protein (MCP-1) in vitro and in vivo.

LPS administration leads to downstream activation of the NF-κB pathway and stimulates the production of IL-6 [22]. IL-6 is an inflammatory cytokine and a fundamental component of the acute phase response, where it is involved in the recruitment of neutrophils to the site of injury [11]. It is thought that IL-6 mediates the switch from acute to chronic inflammation via activation of trans-signaling [23]. IL-6 could be also defined as a pleiotropic cytokine involved in the regulation of immune responses, the acute-phase reaction, and hematopoiesis. In recent studies IL-6 has been shown to suppress inflammation and to play protective role in LPS-galactosamine septic shock model in mice [24].

In our results all groups treated with LPS have increased levels of IL-6 which could be explained by its pro-inflammatory functions during acute phase response.

Considering the ALA anti-inflammatory potential and inhibition on the NF-kB pathway a decrease in the IL-6 levels have to be expected. Li *et al.;* [25] have shown that pretreatment of ALA suppresses TNF-alpha, IL-1β, IL-6, PGE2 levels and inhibits activation of NF-κB signaling pathway in rat mesenglial cells stimulated with LPS.

However, our results are in contrast with the data found in the literature. In our study pretreatment with ALA did not decreased the level of IL-6 in LPS-induced model of inflammation. Moreover, the low dose of the drug showed significantly increase in the level of IL-6 whereas the other two doses showed levels comparable to that of the model group. We could speculate that ALA influences the IL-6 levels in a dose-dependent manner. These finding could be explained by a possible biphasic action of ALA on the IL-6 levels. This is not a unique phenomenon. Franchimont *et al.;* [26] showed increased IL-10 levels using low concentrations of dexamethasone and decreased IL-10 levels to below baseline with higher doses of dexamethasone. Salinthone *et al;* [22] reported decrease in IL-6 levels with ALA treatment in LPS-induced inflammation in vitro. However, they emphasized on the fact that even after the ALA administration a large amount of IL-6 is still produced in response to LPS stimulation.

CONCLUSION

Collectively, the cytokine data suggest that ALA is exerting its anti-inflammatory effects by decreasing TNF-alpha concentrations while possibly having a biphasic effect on IL-6. Our findings showed that chronic administration of alpha-lipoic acid has protective effect in LPS-induced model of inflammation.

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