The effect of lipoic acid on the content of SOD-1 and TNF- α in rat striated muscle

Original Article

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Abstract

Background. The aim of the study was to present the effect of lipoic acid (LA) on oxidative stress induced by lipopolysaccharide (LPS).

Materials/Methods. The studies were conducted on male rats of the Wistar strain. The animals were divided into four groups. I: the controls received saline (0.2 ml); II: LPS, received LPS (*Escherichia coli* O26: B6) at a dose of 6 mg/kg body weight; III: LA, received LA at a dose of 60 mg/kg body weight; IV: LA + LPS, received LA (60 mg/kg b.w.) and after 30 min received LPS (6 mg/kg b.w.). All compounds were administered to the tail vein. After 5 hours of the experiment, the animals were anesthetized and striated muscle from the thigh was prepared. The isolated muscle was homogenized. Concentrations of superoxide dismutase-1 (SOD-1) and tumor necrosis factor alpha (TNF- α) were determined in the homogenates with the application of ELISA.

Results. The study showed a significant decrease in SOD-1 content and an increase in TNF- α in striated muscle after LPS administration. LA given 30 min before administration of LPS caused a significant increase in the level of SOD-1 and decreased levels of TNF- α in homogenates.

Conclusion. LA reduced the parameters of LPS oxidative stress, thus contributing to an increase in the body's antioxidant defense.

Keywords

lipoic acid • oxidative stress • SOD-1 • TNF- α • lipopolysaccharide

Introduction

Sepsis is a systemic inflammatory reaction that develops as a result of infection with bacteria, viruses or fungi. Generalized inflammation is accompanied by excessive generation of reactive oxygen species (ROS), overproduction of pro-inflammatory mediators, and tissue and organ dysfunction as a result of vascular epithelial damage. All this often leads to the death of the body. Sepsis is also the cause of neuromuscular disorders such as critical myopathy, which can lead to muscle atrophy [1, 2].

One of the main factors causing sepsis is lipopolysaccharide (LPS). Lipopolysaccharide is an endotoxin from cell membranes of gram-negative bacteria. LPS is frequently used in animal models of septic shock that are characterized by a fall in blood pressure that is resistant to standard therapy. In the course of septic shock, peripheral vasodilation, edema formation, as well as greatly enhanced flux of macromolecules and fluid from plasma to tissues, are also observed [3]. The injection of LPS can produce a high mortality rate in animals that varies with the LPS dose and

serotype of LPS [4]. In our experiment we used LPS from the *E. coli* serotype O26: B6. This serotype was selected to induce striated muscle inflammation with sepsis, but without risk of mortality. The serotype O26: B6 of LPS from *E. coli* and its effect on oxidative stress was confirmed by other studies [5, 6].

LPS stimulates macrophages to secrete large amounts of inflammatory markers such as tumor necrosis factor α (TNF- α) and interleukin (IL-1, IL-6, IL-8) [7, 8]. High concentrations of TNF- α and IL-6 during endotoxemia induce protein degradation in skeletal muscle, which contributes to muscle atrophy [9]. The nuclear factor NF- κ B plays a major role in the development of sepsis. As a result of binding of LPS to Toll-like receptors (TLR), the NF- κ B factor is separated from its inhibitor. This displaces the NF- κ B factor into the cell nucleus and initiates the expression of pro-inflammatory cytokines and adhesion molecules involved in proliferation, apoptosis, and response to oxidative stress [10]. Many authors have revealed that reactive oxygen species (ROS), formed during

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sepsis, accumulate mainly in mitochondria and are involved in muscle atrophy [11, 12, 13, 14].

ROS may cause DNA damage, lipid peroxidation, protein modification, and activation of the NF- κ B nuclear factor [15, 16, 17] and the erythroid-2 derived nuclear factor (Nrf2) [18].

In order to prevent excessive activation of pro-inflammatory factors, the body produces anti-inflammatory mediators, antioxidant enzymes and other antioxidants that respond to ROS and can stop the development of the inflammatory process. Lipoic acid (LA) is one of the most important endogenous antioxidants. Its synthesis occurs in human mitochondria, but its insufficient quantity cannot maintain antioxidative and pro-oxidative balance; therefore it must be supplied with a diet. From food, it is rapidly absorbed and transported to intracellular compartments, where it is reduced to dihydrolipoic acid (DHLA). Both of these compounds (LA and DHLA) can react with ROS and deactivate them. LA is able to "scavenge" the hydroxyl radical (OH^*), singlet oxygen ($^1\text{O}_2$), hypochlorous acid (HOCl), and peroxynitrite (ONOO^-). However, it does not react with superoxide anion radical (O_2^*) and hydrogen peroxide (H_2O_2). In addition, LA has the ability to chelate heavy metal ions such as: Mn^{2+} , Cu^{2+} , Pb^{2+} , Fe^{2+} , which may also contribute to reducing the effects of oxidative stress. An important role of LA includes regeneration of antioxidants, such as vitamins C and E, and glutathione [19]. LA also affects signaling pathways, and prevents NF- κ B activation induced by TNF- α and phorbol esters [20]. These actions mean that it may be an effective antioxidant against LPS-induced ROS. The purpose of the present study was to assess the content of superoxide-1 dismutase (SOD-1) in rat striated muscle after administration of lipoic acid in the early phase of endotoxic shock.

1. MATERIAL AND METHODS

1.1 Chemicals

Lipopolysaccharide (*Escherichia coli* LPS 026:B6; lyophilized powder chromatographically purified by gel filtration [protein content < 1%]) and α -lipoic acid (solid form) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

All other reagents were obtained from POCH (Gliwice, Poland) and were of analytical grade.

Shortly before use, LPS was dissolved in sterile pyrogen-free 0.9%NaCl. LA was mixed with sterile 0.9%NaCl in a dark bottle and NaOH was then added until the suspension dissolved and the pH was brought to 7.4 with HCl.

Sterile, deionized water (resistance > 18 M Ω cm, HPLC Water Purification System USF ELGA, England) was used throughout the study.

1.2 Animals

The experiments were performed on adult male Wistar rats weighing 250–280 g. The animals were randomly divided into 4 groups and each one consisted of 8 individuals:

Control group I: received 0.2 ml of 0.9% NaCl. Group II-LPS: received LPS (*Escherichia coli* 026: B6) at a dose of 6 mg/kg body weight. Group III-LA: received lipoic acid at a dose of 60 mg/kg body weight. Group IV-LA + LPS: received LA (60 mg/kg) and after 30 min it received LPS (6 mg/kg). All compounds were injected into the tail vein. After 5 hours of experiment, the animals were euthanized by decapitation and the striated muscle from the thigh was dissected.

The experimental procedures were conducted in compliance with the guidelines for the care and use of laboratory animals and approved by the Ethics Committee of the Medical University of Lodz. Resolution No. 7/LB699/2014.

1.3 Tissue homogenization

The isolated muscle was rinsed with cold 0.9% NaCl, dried and weighed. Next, it was cut into 50 mg portions and frozen at -80°C. Biochemical tests were performed within 2 weeks.

Frozen sections (50 mg) were cut into small pieces and homogenized with 2 ml cold PBS solution in a glass homogenizer. All stages were carried out on ice. The finished homogenates were subjected to 2 cycles of freezing (at -70°C) and thawing for further fragmentation of cell membranes. Then, the homogenates were centrifuged for 5 min at 4°C (5000 x g). After centrifugation, the supernatants were collected and frozen at -70°C. The material was subjected to a quantitative analysis with the use of the ELISA test. The test was carried out within 2 weeks.

1.4 Determination of SOD-1 and TNF- α concentrations

To determine the concentration of SOD-1 and TNF- α in rat striated muscle, the authors used ELISA (enzyme-linked immunosorbent assay) from Cloud-Clone Corp No. SEB960RA, US, containing a monoclonal antibody specific for rat TNF- α , SOD-1. The tests were carried out in accordance with the manufacturer's instructions. Spectrophotometric evaluation of the samples was carried out on an EL x 800 Bio-Tech Instruments spectrometer at a wavelength of 450 nm and a correction of 540 nm. Protein concentration for individual parameters was assessed on the basis of a standard curve prepared for increasing concentrations of SOD-1: 12.5; 25; 50; 100; 400; 800 ng/mg and TNF- α , respectively: 12.5; 25; 50; 100; 200 pg/mg. All samples were analyzed in duplicate.

1.5 Statistical Analysis

The data are presented as mean $\pm$ SE from 8 animals in each group. SOD-1 concentration was expressed in ng/ml protein and presented as standard error of the mean (SEM). TNF- α concentration was expressed in pg/ml. Differences between the results in individual groups were assessed using the Student's t test. In

all groups, the significance level $p < 0.05$ was considered statistically significant.

2. RESULTS

The concentrations of SOD-1 and TNF- α in muscle tissue is shown in Table 1. Administration of LPS alone caused a significant decrease in SOD-1 concentration in rat muscle tissue ($p < 0.003$) compared to the control group. Administration of LA alone resulted in an increase in SOD-1 concentration compared to the control group and the LPS group ($p < 0.01$). LA given 30 min before LPS administration caused a marked increase in SOD-1 concentration compared to the LPS group ($p < 0.01$).

LPS challenge caused a marked rise in the levels of TNF- α compared to the control group ($p < 0.001$). Administration of LA or LA + LPS alone resulted in a significant decrease in TNF- α concentration as compared to the LPS group ($p < 0.001$).

3. DISCUSSION

Lipopolysaccharide induces changes in the mitochondrial respiration chain, contributing to lactate production, intracellular acidosis, ROS generation and pro-inflammatory cytokines. Inflammatory cytokines (TNF- α and IL-6) play an important role in muscle wasting and proteolysis [21, 22, 23].

In the present study, the increase in TNF- α concentration after administration of LPS indicates its pro-inflammatory effect, and the development of oxidative stress. High ROS levels may cause skeletal muscle protein degradation, contributing to muscle atrophy [14].

A significant decrease in SOD-1 concentration after LPS administration also indicates an increase in oxidative processes in muscle tissue. Linke et al. showed lessened activity of antioxidant enzymes such as: SOD, catalase (CAT), and glutathione peroxidase in skeletal muscles during oxidative stress [24]. Other authors also observed a reduced level of oxidative stress and a reduced atrophy in skeletal muscle after administration of SOD and CAT or antioxidant vitamins (vitamin E) [25, 26, 27]. Besides, ROS may activate the nuclear factor kappa B (NF- κ B) and many stress-sensitive cell pathways such as JNK kinase phosphorylation (c-Jun N-terminal kinase) [28]. Oxidative cell damage, caused by LPS, is facilitated by impaired function of endogenous antioxidant enzyme [29, 30]. A decrease in the activity of endogenous antioxidant enzymes causes an increase in the activity of stress-sensitive cell pathways, such as NF- κ B [31]. A body has defense

mechanisms that protect skeletal muscles from damage caused by ROS. These include antioxidant enzymes, e.g. superoxide dismutase, but also glutathione and thioredoxin [32]. The family of SOD enzymes (cytoplasmic SOD-1 – Cu/ZnSOD, mitochondrial SOD-2 – MnSOD and extracellular – EcSOD) catalyzes deactivation of the toxic peroxide anion to hydrogen peroxide and oxygen [33].

Lipoic acid is a powerful antioxidant that can “scavenge” many reactive oxygen species, including peroxide, hydroxyl radical, and peroxide radical [34, 35]. This compound has the ability to chelate metal ions, and regenerate ascorbate and tocopherol. It also increases concentrations of SOD and glutathione [36, 37, 38].

In the present study, the increased level of SOD-1 after LA may be associated with a decrease in the ROS level. Similarly, Zhao et al. showed an increase in Cu/ZnSOD mRNA expression concentration in the human ARPE-19 cell line after LA [39].

Another study demonstrated that LA possessed preventive and therapeutic effects on endotoxic shock in rats. LA effectively attenuated LPS-induced acute inflammatory response and improved multiple organ function in endotoxemia. Shen et al. suggest that the antioxidant and anti-inflammatory effects of LA accounted for reducing proinflammatory cytokines and increasing the survival rate of endotoxemic rats [40].

LA has been shown to inhibit NF- κ B activation in lung tissue in a cecal ligation and a puncture-induced sepsis model, and to reduce serum proinflammatory cytokines TNF- α and IL-6 levels [41].

In our study, we revealed that treatment with LA can suppress LPS-induced TNF- α release. Shen et al. obtained the same results in their experimental model on multiple organ dysfunction syndrome (MODS) [40]. It has been suggested that lipoic acid is able to attenuate mortality or prevent organ damage in LPS-induced endotoxemic animal models [42].

Clinical trials of antioxidant supplementation (lipoic acid, melatonin, N-acetylcysteine, vitamins, carnitine) reduced the incidence of septic shock and prevented organ damage in oxidative stress [43, 44, 45].

Our study furthers the understanding of preventive and therapeutic effects of LA on oxidative stress. These results also showed the promising potential of LA in preventing the development of the inflammatory process in oxidative stress. The

Table 1. The influence of administration LA on SOD-1 and TNF- α content in striated muscle of rats subjected to LPS-induced oxidative stress

Parameter	Saline	LPS	LA	LA+LPS
SOD-1 (ng/mg protein)	53.9 $\pm$ 1.74	39.4 $\pm$ 1.87 *#	65.3 $\pm$ 3.82	60.5 $\pm$ 2.39
TNF- α (pg/mg protein)	23.17 $\pm$ 1.23	66.88 $\pm$ 1.3 *	25.4 $\pm$ 1.8	38.2 $\pm$ 2.4

* $p < 0.001$ vs saline, LA and LA+LPS; # $p < 0.01$ vs LA, LA+LPS; LPS- lipopolysaccharide, LA-lipoic acid, SOD-1 superoxide dismutase-1, TNF- α – tumor necrosis factor alpha.

detailed mechanism of action of LA needs to be further investigated.

4. CONCLUSIONS

Lipoic acid, administered 30 min before causing oxidative stress, increases the concentration of superoxide dismutase and reduces the concentration of tumor necrosis factor α in striated muscle, thus enhancing the body's antioxidant defense.

Authors' Contribution

B.S.: carrying out the experiments, acquisition of data, data analysis and interpretation, writing- original draft preparation; **A.S.:** acquisition of data, writing- review and editing, literature review, visualization; **A.G.:** research concept and design, supervising the project, final proofreading and approval of the version for publication.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

Ethics Approval

The experimental procedures were conducted in compliance with the guidelines for the care and use of laboratory animals and approved by the Ethics Committee of the Medical University of Lodz. Resolution No. 7/LB699/2014.

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