Research paper

α-Lipoic acid attenuates LPS-induced liver injury by improving mitochondrial function in association with GR mitochondrial DNA occupancy

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ABSTRACT

α-Lipoic acid (LA) has been demonstrated to be a key regulator of energy metabolism. However, whether LA can protect the liver from inflammation, as well as the underlying mechanism involved, are still largely unclear. In the present study, mice treated with lipopolysaccharide (LPS) and injected with LA were used as a model. Liver injury, energy metabolism and mitochondrial regulation were investigated to assess the protective effect of LA on the liver and explore the possible mechanisms involved. Our results showed that LA attenuated liver injury, as evidenced by the decreased plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels after LA treatment compared with the LPS-treated group. The hepatic ATP and NADH levels, expression levels of most mitochondrial DNA (mtDNA)-encoded genes as well as mitochondrial complex I, IV and V activities were all significantly increased in the LA-treated group compared with the LPS-treated group. Levels of Sirt3 protein, which is essential for the regulation of mitochondrial metabolism, were also increased in the LA-treated group. Regarding the regulation of mtDNA-encoded genes expression, we observed no obvious change in the methylation status of the mtDNA D-loop region. However, compared to the LPS-treated group, LA treatment increased glucocorticoid receptor (GR) protein expression in the liver, as well as the level of GR occupancy on the mtDNA D-loop region. Our study demonstrates that LA exerts a liver-protective effect in an inflammation state by improving mitochondrial function. Furthermore, to the best of our knowledge, we demonstrate for the first time that GR may be involved in this effect via an enhanced binding to the mtDNA transcriptional control region, thereby regulating the expression of mtDNA-encoded genes.

1. Introduction

The liver is often exposed to systemic infectious pathogens, including hepatotropic and nonhepatotropic microorganisms. These pathogens can directly or indirectly cause liver injury. It has been demonstrated that inflammation may induce a massive loss of hepatocytes and exacerbate the severity of various hepatic conditions, which are associated with irreversible liver damage, fibrosis, and carcinogenesis [1–3]. In addition, most hepatic disorders are characterized by profound mitochondrial dysfunction that can either drive massive hepatocyte death [4] or sensitize them to a variety of otherwise non-lethal stress conditions, such as hypoxia and/or nutrient shortage [5]. Indeed, mitochondria regulate energy homeostasis by controlling cellular fuel oxidation and ATP production. Structural and functional mitochondrial alterations have been reported to accumulate in livers affected by insulin resistance injury, nonalcoholic fatty liver, sepsis and Wilson's disease [5–7].

α-Lipoic acid (1,2-dithiolane-3-pentanoic acid; LA), a naturally occurring short chain fatty acid, is a necessary cofactor for the mitochondrial pyruvate dehydrogenase and α-ketoglutarate

dehydrogenase complexes, therefore playing a critical role in mitochondrial energy metabolism [8]. It was previously reported that LA improves mitochondrial function by stimulating sirtuin 1 and 3 [9]. Although LA has been described as a potent biological antioxidant that protects tissues during inflammation [3,10], it is still unclear whether LA protects the liver from inflammation by improving mitochondrial function. Importantly, mitochondrial DNA (mtDNA) encodes for 13 proteins, and its D-loop region, which is analogous to the promoter of nuclear DNA-encoded genes, controls gene transcription [11]. However, it remains largely unknown whether LA can improve liver mitochondrial function by acting on the mitochondrial D-loop region during inflammation.

Glucocorticoids (GCs) are important regulators of whole body energy homeostasis [12], and their levels increase quickly in response to inflammation [13]. The glucocorticoid receptor (GR) plays a significant role in the anti-inflammatory effects of GCs on target tissues [14]. GR acts as a transcription factor for distinct target genes, regulating them through both direct DNA binding and protein–protein interactions with other transcription factors [15]. It has been confirmed that GR is involved in the inflammatory response and plays an anti-inflammatory role [16,17]. GR was previously shown to translocate into the mitochondria and bind to the glucocorticoid response elements located in the mtDNA D-loop region [18,19], thereby exerting measurable effects on mitochondrial function [20]. However, the participation of GR in the LA-mediated hepatic mitochondrial function effects during inflammation has never been investigated.

Lipopolysaccharide (LPS) treatment is a prototypical, well-studied trigger of the inflammatory response and represents the mainstay of experimental inflammation models [21]. In the present study, we pretreated mice with LA and injected them with LPS to investigate the effects of LA on the liver energy metabolism, mitochondrial function, and inflammatory response. We also investigated the role of GR in the LA-mediated mitochondrial function effects during inflammation. Our results will help to elucidate the effects of LA on liver metabolism during inflammation and further clarify their underlying mechanisms.

2. Materials and methods

2.1. Ethics statement

Experiments were conducted in accordance with the guidelines of the Animal Ethics Committee of Nanjing Agricultural University, China. Euthanasia and sampling procedures complied with the "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398 published by the Ministry of Science and Technology, China, and with the "Regulation regarding the Management and Treatment of Experimental Animals" (2008) No. 45, published by the Jiangsu Provincial People's Government.

2.2. Animals and experimental design

Six-week-old male C57BL/6 mice (n = 44) weighing 23.79 ± 0.12 g were obtained from the Comparative Medicine Centre of Yangzhou University (Yangzhou, China, certificate of quality is SCXK (Su) 2012-0004). Animals were housed in a temperature-controlled room (22 ± 2 °C) with a 12:12-h light–dark cycle. Mice were fed a pellet chow diet and given deionized water ad libitum over a 5-day adaptation period. After this period, mice were assigned to three experimental groups for five more days. On the fifth day, the control group (n = 12) received an intraperitoneal injection of saline and the LPS group (n = 16) received LPS dissolved in saline at a dose of 5 mg/kg Escherichia coli 055:B5 LPS (L2880, Sigma–Aldrich). The LA + LPS group (n = 16) was co-treated with LPS and (±)-α-Lipoic acid (T1395, Sigma–Aldrich). In the LA + LPS group, LA (dissolved in saline with NaOH, and the pH was neutralized with HCl, and sterilely filtering the resulting solution) was administered intraperitoneally at a dose of 100 mg/kg daily, and on the fifth day, LPS was injected 1 h after the last LA injection, at a dose of 5 mg/kg. Six hours after the LPS injection, half of each group (6 mice in control group and 8 mice in both LPS and LA + LPS group, respectively) was randomly selected and anesthetized for blood collection from the inferior vena cava. The liver (without the gall bladder) was then weighed and immediately frozen in liquid nitrogen prior to storage at −70 °C until further analysis. Twenty-four hours later, the remaining mice were processed in the same way. All mice were starved for 2 h before sampling. Body weight was recorded before the first experimental manipulations and at time of sampling.

2.3. Plasma biochemical index analysis

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, triglyceride and total cholesterol levels were analysed using an automatic biochemical analyser (Olympus AU2700) at the Nanjing General Hospital of Nanjing Military Command (Nanjing, China).

2.4. Measurement of plasma corticosterone and tumour necrosis factor-α (TNF-α) concentrations

Plasma corticosterone concentrations were determined using a corticosterone ELISA kit according to the manufacturer's protocol (ADI-900-097, Assay Designs Inc., Ann Arbor, MI, USA). Plasma TNF-α concentrations were measured using a commercially available ^{125}I-RIA kit (Technology Research Institute of the Northern biological Inc., Beijing, China). Antibodies cross-reactivity, detecting potentially competing plasma steroids in the kits, was lower than 10%. Corticosterone and TNF-α assay sensitivity was 27.0 pg/mL and 6.0 fmol/mL, respectively.

2.5. Real-time RT-PCR for mRNA quantification

Total RNA was isolated from liver samples using TRIzol Reagent (no. 15596026, Invitrogen) according to the manufacturer's instruction. Total RNA extracts were treated with DNase I (D2215, Takara) to eliminate any possible genomic DNA contamination. Two micrograms of total RNA were reverse transcribed and 2 μL of diluted cDNA (1:20) were used for real-time PCR analysis. Peptidylprolyl isomerase A (PPIA) was chosen as a reference gene. All primers were synthesized by Generay Biotech and are listed in Table 1. The $2^{-\Delta\Delta Ct}$ method was used for real-time PCR data analysis.

2.6. Western blotting for protein quantification

Liver samples were homogenized in RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% NP40, 0.25% Na-deoxycholate, 1 mM PMSF, 1 mM sodium orthovanadate with Roche EDTA-free complete mini protease inhibitor cocktail, no. 11836170001) to get the total proteins. Nucleoproteins were extracted using a nuclear and cytoplasmic protein extraction kit (803051, Biouniquer). Proteins concentrations were determined using a Pierce BCA Protein Assay kit (23225, Thermo). Western blot analysis of target proteins was carried out according to the protocols provided by the primary antibody suppliers. β-actin was selected as loading control. The anti-GR (sc-1004X, Santa Cruz, 1:200), anti-PGC-1α (sc-13067, Santa Cruz, 1:200) and anti–NF–κB (sc-109, Santa Cruz, 1:200) antibodies were purchased from Santa Cruz Biotechnology. The anti-Sirt3 antibody (ab86671, Abcam, 1:1000) was purchased from