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Alpha-lipoic acid exerts a liver-protective effect in acute liver injury rats

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ABSTRACT

Background: Recent evidence indicates that alpha-lipoic acid (α-LA) has a variety of liver-protective effects through the suppression of inflammatory mediators including tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS). However, there are few reports that α-LA markedly enhanced the survival rate in animal models of liver injury with more than 90% death. The aim of this study was to investigate the beneficial effects of α-LA in a rat model of acute liver injury and to clarify the mechanisms of α-LA action.

Methods: Rats were treated with D-galactosamine and lipopolysaccharide (GalN and LPS) to induce acute liver injury. α-LA (100 mg/kg) was administered intraperitoneally 1 h before GalN and LPS injection. Inflammatory mediators including TNF-α and iNOS were analyzed.

Results: A single injection of α-LA improved the survival rate by more than 80%. α-LA prevented serum transaminase increases, histopathologic changes, and apoptosis in the liver. In the serum, α-LA decreased TNF-α production and increased interleukin (IL)-10 production. In the liver, α-LA reduced TNF-α and IL-6 messenger RNA (mRNA) but enhanced IL-10 mRNA. α-LA decreased the expression of iNOS mRNA and its antisense transcript, leading to the reduction of iNOS protein expression and resulting in the inhibition of nitric oxide production. An electrophoretic mobility shift assay revealed that α-LA reduced the activation of nuclear factor-kappa B induced by GalN and LPS.

Conclusions: α-LA inhibited the induction of inflammatory mediators, such as TNF-α and iNOS, in part through the inhibition of nuclear factor-kappa B activation and enhanced the induction of IL-10. α-LA may have therapeutic potential for use in the prevention of acute liver injury.

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1. Introduction

Alpha-lipoic acid (α-LA), which is present in all types of prokaryotic and eukaryotic cells, is a key regulator of energy metabolism in mitochondria [1]. α-LA has been used as a therapeutic agent in the treatment of diabetic neuropathy [2] and as a nutritional supplement, which does not have serious side effects, in many countries. In addition to its well-

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described antioxidant effects, α-LA exhibits distinct regulatory action on signal transduction processes playing a central role in tissue damage and protection [3–5]. In this context, α-LA has been described as a therapeutic agent in a number of conditions related to liver disease, including alcohol-induced damage, metal intoxication, and hyperdynamic circulation in biliary cirrhosis [6–8].

D-Galactosamine (GalN) has liver specificity because hepatocytes have high levels of galactokinase and galactose-1-uridyltransferase. GalN-induced liver damage is recognized to be similar to human viral hepatitis from both metabolic and morphologic aberrations [9,10]. GalN has often been used in combination with lipopolysaccharide (LPS) to produce an animal model of severe acute liver injury (more than 90% of death). In this model, the toxic effects of D-galactosamine and lipopolysaccharide (GalN and LPS) are mediated through the generation of endogenous proinflammatory cytokines, including tumor necrosis factor (TNF)-α [11,12]. LPS and proinflammatory cytokines, such as TNF-α and interleukin (IL)-1β, stimulate the induction of inducible nitric oxide synthase (iNOS) gene expression, which is followed by the enhancement of nitric oxide (NO) production. High levels of NO induced by iNOS have been implicated as one of the factors in liver injury, although NO has been reported to exert either detrimental or beneficial effects depending on the insults and tissues involved.

In the liver, the activation of Kupffer cells represents a central mechanism of inflammatory liver injury involving the production of two important inflammatory mediators, namely TNF-α and NO. Kiemer *et al.* [13] reported that α-LA inhibits LPS-induced NO and TNF-α production in rat Kupffer cells. Suntres *et al.* [14] found that the pretreatment of α-LA results in a significant alleviation of liver injuries in an LPS-induced acute organ injury model of rats. Shanmugarajan *et al.* [15] reported that α-LA influences the antioxidant status in GalN-induced hepatic injury. These reports indicate that α-LA may have potential effects on the prevention of various liver injuries.

However, there are few reports that α-LA markedly enhanced the survival rate in animal models of acute liver injury with more than 90% death. In this study, we investigated whether the treatment of α-LA improves the survival rate in GalN and LPS-treated rats. Furthermore, we examined whether α-LA influences the expression of various inflammatory mediators including TNF-α and iNOS, and anti-inflammatory cytokine, IL-10, to clarify the mechanisms of α-LA action involved in the liver-protective effects.

2. Materials and methods

2.1. Materials

α-LA (1,2-dithiolane-3-pentanoic acid) was purchased from Sigma Chemical Co (St. Louis, MO). GalN hydrochloride and LPS (Escherichia coli 0111: B4) were purchased from Wako Pure Chemical (Osaka, Japan).

2.2. Experimental design

Male Sprague–Dawley rats obtained from Charles River (Tokyo, Japan) were kept at 22°C under a 12-h light–dark cycle and received food and distilled water *ad libitum*. After at least 7 d of acclimation to these conditions, rats (280–320 g and 8-wk old) were anesthetized with isoflurane (Abbott Laboratories, Abbott Park, IL) before injection. GalN and LPS (500 mg/kg GalN plus 50 μg/kg LPS) were injected into the penile vein as reported previously [16]. α-LA (100 mg/kg) or saline was administered intraperitoneally 1 h before GalN and LPS injection. The survival was checked during the 72 h after the injection of GalN and LPS. Blood and liver samples were obtained from individual rats at the indicated times. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Animal Care Committee of Kansai Medical University.

2.3. Biochemical analysis

Blood samples were collected 1, 3, and 6 h after the GalN and LPS injection. Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using commercial kits (Wako Pure Chemical, Osaka, Japan). TNF-α, IL-6, IL-10 (R&D Systems, Minneapolis, MN), and cytokine-induced neutrophil chemoattractant (CINC)-1 (human IL-8 analog) (Enzo Life Sciences, Plymouth Meeting, PA) were measured in serum using commercial kits.

2.4. Histopathologic analysis

Excised liver specimens that were taken 12 h after the GalN and LPS treatment were fixed in 10% formalin and embedded in paraffin. Sections (3–5 μm) were cut and stained with hematoxylin and eosin. Apoptotic bodies were detected by terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-digoxigenin nick-end labeling (TUNEL) staining using an apoptosis detection kit (Medical and Biological Laboratories Co, Nagoya, Japan). The TUNEL-positive and TUNEL-negative cells were counted in randomly selected fields of view under light microscopy (×100). The mean number of TUNEL-positive cells was used to calculate the apoptotic index (%) (number of apoptotic cells/total number of cells × 100).

2.5. Serum nitrite and/or nitrate analysis

The sum of serum nitrite and nitrate (NO_2^- and NO_3^-, respectively; stable metabolites of NO) was measured using the Griess reagent method using a commercial kit (Roche Diagnostics, Mannheim, Germany) [17].

2.6. Western blot analysis

Frozen liver samples were homogenized in five volumes of cell solubilizing buffer (10 mM Tris-HCl, pH 7.4; containing 1% Triton X-100 (Wako Pure Chemical, Tokyo, Japan), 0.5% Nonidet P-40 (Wako Pure Chemical), 1 mM ethylenediaminetetraacetic acid [EDTA], 1 mM ethylene glycol bis [2-aminoethyl ether] tetraacetic acid, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride [PMSF], and protease inhibitor cocktail [Roche Diagnostics]) and centrifuged (16,500*g* for 15 min). The supernatant was mixed with sodium dodecyl

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