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Original Article

Long-chain bases from sea cucumber mitigate endoplasmic reticulum stress and inflammation in obesity mice

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ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form
9 October 2016
Accepted 17 October 2016
Available online 29 November 2016

Keywords:
long-chain bases
sea cucumber
endoplasmic reticulum stress
inflammation
cytokines

ABSTRACT

Endoplasmic reticulum (ER) stress and inflammation can induce hyperglycemia. Long-chain bases (LCBs) from sea cucumber exhibit antihyperglycemic activities. However, their effects on ER stress and inflammation are unknown. We investigated the effects of LCBs on ER stress and inflammatory response in high-fat, fructose diet-induced obesity mice. Reactive oxygen species and free fatty acids were measured. Inflammatory cytokines in serum and their mRNA expressions in epididymal adipose tissues were investigated. Hepatic ER stress-related key genes were detected. c-Jun NH_2-terminal kinase and nuclear factor κB inflammatory pathways were also evaluated in the liver. Results showed that LCBs reduced serum and hepatic reactive oxygen species and free fatty acids concentrations. LCBs decreased serum proinflammatory cytokines levels, namely interleukin (IL)-1β, tumor necrosis factor-α, IL-6, macrophage inflammatory protein 1, and c-reactive protein, and increased anti-inflammatory cytokine IL-10 concentration. The mRNA and protein expressions of these cytokines in epididymal adipose tissues were regulated by LCBs as similar to their circulatory contents. LCBs inhibited phosphorylated c-Jun NH_2-terminal kinase and inhibitor κ kinase β, and nuclear factor κB nuclear translocation. LCBs also inhibited mRNA expression of ER stress markers glucose regulated protein, activating transcription factor 6, double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase, and X-box binding protein 1, and phosphorylation of eukaryotic initiation factor-α and inositol requiring enzyme 1α. These results indicate that LCBs can alleviate ER stress and inflammatory response. Nutritional supplementation with LCBs may offer an adjunctive therapy for RE stress-associated inflammation.

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http://dx.doi.org/10.1016/j.jfda.2016.10.011

1. Introduction

Low-grade inflammation in adipose tissue is considered an important procedure in the development of obesity-related comorbidities, including diabetes mellitus, hyperglycemia, and insulin resistance [1,2]. The chronic inflammation in adipose tissue is powerfully augmented through the secretion of free fatty acids (FFAs) and deleterious inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α) [3]. Elevated proinflammatory cytokines and excessive oxidative stress maintain a proinflammatory environment, and firstly cause tissue damage in the liver and lead to further activation of inhibitor κ kinase β (IκKβ), c-Jun NH_2-terminal kinase (JNK), and other serine kinases [4]. These serine kinases, in turn, lead to the production of IL-1β, TNF-α, FFAs, reactive oxygen species (ROS), etc. [5,6]. Recent studies suggested that endoplasmic reticulum (ER) stress was an important contributor to chronic tissue inflammation [7,8]. ER is the organelle for the synthesis, folding and trafficking of secretory and membrane proteins [9]. Disruption of ER homeostasis results in an adaptive unfolded protein response (UPR) triggered by glucose regulated protein (GRP78/Bip) pathway, intended to restore the capacity of ER and alleviate this stress [10]. Three distinct ER transmembrane proteins initiate the canonical UPR: activating transcription factor 6 (ATF6), inositol requiring enzyme 1 (IRE1), and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) [4,11]. Once the UPR is unable to prevent the accumulation of unfolded proteins, ER stress response occurs. JNK and nuclear factor (NF) κB pathways are directly involved in ER stress-induced inflammatory response. For example, JNK and NFκB can be activated by IRE1 through the pro-inflammatory cytokine, TNF-α [12]. phosphoenolpyruvate kinase/eukaryotic initiation factor α (eIF2α)-triggered ER stress directly promotes NFκB nuclear translocation [13], which subsequently increases the productions of TNF-α and IL-6 [14].

Long-chain bases (LCBs), also called sphingoid bases, are the simplest members in the family glycosphingolipids. Current studies showed that LCBs possessed several bioactivities, such as antioxidation, antitumor, improvement in type 2 diabetes, and inhibition of keratinocyte differentiation [15–18]. LCBs from marine organisms exhibit better bioactivities because of their special environment [19]. Therefore, raising contents have been drawn in LCBs extracted from sea cucumbers (SC-LCBs). SC-LCBs were reported to induce apoptosis in human hepatoma HepG2 cells through phosphorylated protein kinase B and death receptor-5 [20]. SC-LCBs also ameliorated glucose tolerance and hepatic triglyceride content in obese mice [21,22]. However, the effects of LCBs on ER stress associated with inflammatory response have not been verified. Sphingoid bases from plants were proved to inhibit TNF-α and IL-8 levels in human endothelial cells, but the mechanism is not understood [23]. Therefore, the present study was conducted to evaluate whether LCBs could influence ER stress and inflammation or not. In addition, the molecular mechanism by which LCBs altered ER stress-induced inflammatory signal transduction was also investigated.

2. Methods

2.1. Preparation of LCBs

Dried sea cucumber, *Cucumaria frondosa*, was purchased from Nanshan Aquatic Products Market (Qingdao, China). LCBs were extracted and analyzed as the previous reports [24]. Briefly, total lipids were extracted from sea cucumber using chloroform–methanol (2:1 v/v); 4M KOH in methanol was added into the total lipids and performed 2 hours at 37°C. Extraction was subsequently performed with chloroform–methanol–distilled water (2:1:0.9 v/v/v), and the chloroform layer was collected. After vacuum concentration, the lipids were under HCl acidolysis 16 hours at 80°C, and then was extracted using *n*-hexane and subsequently diethyl ether, respectively. The crude LCBs were obtained from diethyl ether. To gain pure LCBs, HPLC was performed using an Agilent 1100 HPLC system (Santa Clara, CA, USA) with diode array detection, and a TSK gel ODS-80Ts. The purity of LCBs was 96.4% using HPLC system with a diode array detector. The yield of LCBs was about 1.47%. Their molecular weights were in the range of 238.4–320.5 analyzed by the electrospray ionization-MS method. The components and the main chemical structure of *C. frondosa* LCBs are shown in Figure 1.

2.2. Animal experiments

Five-week-old male C57BL/6J mice (licensed ID: SCXK2011-0011) were obtained from Vital River Laboratory Animal Center (Beijing, China). Animals ($n = 12$/group) were housed in individual cages under a 12-hour light/dark cycle at 23 ± 1°C daily. The animals were assigned to four groups: control group (maintained on a control diet for 12 weeks); HFFD group [fed high-fat fructose diet (HFFD) diet for 12 weeks]; and low and high dosage of LCBs groups (fed HFFD diet for 4 weeks, and then continuously fed HFFD diet with LCBs at a diet supplement dosage of 0.008% and 0.025% for 8 weeks, respectively). The control diet consisted of 20% protein, 5% fat, and no fructose, whereas the HFFD consisted of 20% protein, 25% fat, and 20% fructose. All experimental protocols used in this study were approved by animal ethics committee as per the guidelines of the Standards for Laboratory Animals of China (GB 14922-94, GB 14923-94, and GB/T 14925-94).

2.3. Fasting serum ROS, FFAs, and inflammatory factors assay

Serum ROS, FFAs, TNF-α, C-reactive protein (CRP), macrophage inflammatory protein 1 (MIP-1), IL-1β, IL-6, and IL-10 levels were assessed with their corresponding enzyme-linked immunosorbent assay kits (Invitrogen, Carlsbad, CA, USA).

2.4. Hepatic ROS and FFAs concentrations analysis

Hepatic ROS and FFAs levels were detected as in our previous study [4]. Briefly, the liver was homogenized in hydroxyethylpiperazine ethane sulfonic acid (HEPES) buffered saline